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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Application of)
)
JEFFRY W. KREAMER) Group Art Unit 1205
)
Serial No. 08/071,052) Examiner: T. Criares
Filed: June 4, 1993)
For: ASPIRIN AND VITAMIN)
AND/OR TRACE ELEMENT)
COMPOSITIONS FOR THE)
PREVENTION AND)
TREATMENT OF VASCULAR)
DISEASE)

93-4911

BRIEF OF APPELLANT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

This is an appeal from the final rejection of the Examiner dated May 18, 1994, rejecting claims 11-26, all of the remaining claims in the case. This Brief is accompanied by the requisite fee set forth in 37 C.F.R. §1.17(f).

Status of Claims (37 C.F.R. §1.92(c)(1))

The present application was filed on June 4, 1993, and is a continuation of application Serial No. 07/746,615, filed August 19, 1991, which is a continuation of application Serial No. 07/317,422, filed March 1, 1989. The present application was filed with ten (10) claims of which six (6) were independent claims (claims 1, 2, 5, 6, 7 and 9).

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All of the claims were rejected by the Examiner in the Office Action dated September 10, 1993.

In Applicant's response dated March 10, 1994, claims 1-10 were deleted and claims 11-26 were added.

The Examiner in the next Office Action dated May 18, 1994, finally rejected all of the remaining claims (claims 11-26). The Examiner granted a personal interview with Applicant's representatives Kent Herink and Brett Trout on November 19, 1993, and with Kent Herink on January 7, 1994. The Examiner also granted Applicant and Applicant's representative Brett Trout a telephonic interview with the Examiner and Supervisory Examiner Marianne M. Cintins on June 2, 1994. No agreement, however, was reached during any of the aforementioned interviews.

The status of the claims as set out in the Examiner's final Office Action of May 18, 1994, was and is as follows:

Allowed claims--None.

Claims objected to--None.

Claims canceled--1-10.

Claims rejected--11-26.

Status of Amendments (37 C.F.R. §1.192(c)(2))

Applicant's amendments of October 13, 1994, have not been entered. The Examiner has stated that these amendments raise new issues that would require further consideration and/or search and that an additional claim is presented without canceling a corresponding number of finally

rejected claims. Accordingly, the claims as set out in the Appendix to this Brief do not include the amendments of October 13, 1994.

Summary of the Invention (37 C.F.R. §1.192(c)(3))

Applicant, Dr. Jeffry Kreamer, has invented a method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans through the reduction of cholesterol incorporation into the endothelium. It is known in the prior art to reduce arteriosclerotic plaque formation at sites of endothelial damage in humans by irreversibly blocking platelet prostaglandin function through the administration of aspirin. The blockage of platelet prostaglandin function inhibits the ability of platelets to go to the site of the endothelial damage and start wound healing by the activity of platelets. Since the platelet activity is reduced, cholesterol migration into the wound is also reduced due to a connection between platelet activity and cholesterol migration. Therefore, plaque formation from cholesterol migration is also reduced, decreasing the likelihood of myocardial infarction to the patient. One drawback of aspirin is that it also decreases prostaglandin function in the vascular wall. Prostaglandins in the vascular wall act to expel cholesterol and inhibit the undesirable plaque formation. While the reduced plaque formation from the reduction of platelet prostaglandin function is greater than the undesirable reduced activity of prostaglandins in the vascular wall, it would be desirable to overcome the plaque formation due to the reduced prostaglandin activity in the vascular wall. The inventive method of the present application involves overcoming

the reduced prostaglandin function in the vascular wall by reducing the amount of cholesterol migrating to the wound and forming plaque material. By orally administering particular vitamins and trace elements in conjunction with aspirin to reduce the amount of cholesterol migrating to the wound site or to speed the wound healing to give the cholesterol less time to migrate to the site, an unexpectedly synergistic, rather than additive, advantage is obtained. In contrast to the detrimental effect anticipated by the attached University of California study (Applicant's Ex. 1), the resulting combination delivers unexpectedly beneficial results. Specifically, the present invention involves a method for blocking prostaglandin function in platelets through the oral administration of aspirin and reducing the migration of cholesterol into the endothelium through the oral administration of a medicament, specifically a vitamin or trace element selected from the group consisting of vitamin A, vitamin B_6, vitamin C, vitamin E, niacin, chromium, selenium, zinc, iron, copper, cobalt, and magnesium.

Issues (37 C.F.R. §1.192(c)(4))

1. The Examiner has objected to the specification under 35 U.S.C. §112, first paragraph, arguing that the proportions of active agents present in the synergistic compositions are not clearly set forth in the specification. The Examiner states that the specification does not set forth what ratios of active agents will yield the desired synergistic effect. The Examiner states that there is no clear teaching of the proportions needed to effect the synergistic activity.

2. The Examiner has rejected claims 11-26 under 35 U.S.C. §112, first paragraph, for the reasons set forth in the objection to the specification. The Examiner argues that claims 11-26 failed to recite the active agents or the ratio of active agents which will yield the synergistic effect. The Examiner argues that the claims are not commensurate with the scope of the data since the data does not teach that each of the medicaments claimed in claims 11-26 are effective individually or in a prescribed combination.

3. The Examiner rejects claims 11-26 under 35 U.S.C. §103 as being unpatentable over Igarashi, et al. '778, Fratzer '603, and Frisbee '081. The Examiner argues that Igarashi, et al. and Fratzer each teach the use of vitamin E to treat vascular disease, and that Frisbee discloses that aspirin can be used to treat vascular diseases. The Examiner states that the use of the two ingredients in a single combination would have been obvious to those skilled in the art given the known characteristics of each component. The Examiner states that the effects on the prostaglandin function and migration of cholesterol would be inherent in the individual administration of the claimed active agents.

Grouping of Claims (37 C.F.R. §1.192(c)(5))

As to the 35 U.S.C. §112 rejection applied against claims 11, 12, 13, 14, 15, 16, 19, 20, 21 and 25, it is Applicant's intention that each of these claims stand or fall independently of one another and of all other claims.

As to the 35 U.S.C. §112 rejection applied against claims 17 and 18, it is Applicant's intention that these claims stand or fall together.

As to the 35 U.S.C. §112 rejection applied against claims 22, 24 and 26, it is Applicant's intention that these claims stand or fall together.

As to the 35 U.S.C. §103 rejection applied against claims 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26, it is Applicant's intention that each of these claims stand or fall independently of one another and of all other claims.

Argument (37 C.F.R. §1.192(c)(6))

ISSUE 1: The objection to the specification under 35 U.S.C. §112.

The Examiner states that since the proportions of active agents present in the synergistic compositions are not clearly set forth in the specification, the specification does not meet the requirements of 35 U.S.C. §112. Applicant, Dr. Kreamer, respectfully submits that dose ranges for aspirin are well known in the art, as are dose ranges for the claimed medicaments which include vitamin A, vitamin B, vitamin C, vitamin E, niacin, selenium, zinc, iron, copper, cobalt, and magnesium.

Witt, et al., U.S. Patent No. 5,093,325, which was cited by the Examiner, states in column 4, lines 18-20, that typically, acetylsalicylic acid (aspirin) is administered, as an individual substance, in an amount of 50-500 milligrams per patient per day. Based upon the level of skill in the art at the time Dr. Kreamer's application was filed, a practitioner of ordinary skill in the art would be able to administer an effective dose of aspirin to block prostaglandin function in platelets sufficiently to reduce the ability of platelets to go to the site of endothelial damage and to recruit other cells to

assist in thrombosis. As noted in the article by Harker, L.A., Circulation 73 (2): 206-223 (1986) (Applicant's Ex. 2) and incorporated into Dr. Kreamer's specification by reference, aspirin dosages for patients are well known in the art with examples ranging from 300 milligrams per day to 1.5 grams per day being given. Id. at 213t. Because an appropriate dosage depends upon a particular patient's age, sex, weight, total diet, vascular condition, blood clotting ability, other medications, and other medical conditions, no specific dosage would constitute a preferred or best mode for all patients to achieve the benefits of Dr. Kreamer's invention. Instead, it is left to the skill of the ordinary practitioner to determine an appropriate dosage of aspirin, based upon a particular patient's aforementioned characteristics, to block prostaglandin function in that patient's platelets sufficiently to reduce the ability of that patient's platelets to go to the site of endothelial damage and to recruit other cells to assist in thrombosis.

Similarly, examples of vitamin B_6 dosages are provided in the Simonson, E., et al., Circulation 24: 1239-1248, 1243 (1961), (Applicant's Ex. 3), which is also incorporated into Applicant's specification by reference. The Simonson reference gives examples of vitamin B_6 doses (delivered as pyridoxine) ranging from 50 to 200 milligrams per patient per day. The Simonson reference also gives example dosages of vitamin C ranging from 500 to 1,000 milligrams per patient per day and dosages of niacin (delivered as nicotinic acid) ranging from 1,000 to 2,000 milligrams per patient per day. Id. at 1243. Typical dosages of the aforementioned vitamins and of all the

remaining vitamins and trace elements may be easily found by one of ordinary skill in the art by reference to readily available materials in both medical libraries and public libraries. Examples of such reference materials are attached herewith and discussed below.

The Right Dose: How to Take Vitamins & Minerals Safely, Patricia Hausman, M.S., Rodale Press, Emmaus, Pennsylvania 1987 (Applicant's Ex. 4), lists dose ranges as follows: Vitamin A - 1,400 to 5,000 I.U. (USRDA 5,000 I.U.); Vitamin B_6 - .3 to 2.6 mg./day (USRDA 2 mg.); Vitamin C - 35 to 100 mg./day (USRDA 60 mg.); Vitamin E - 3 to 11 mg./day (USRDA 30 I.U.); Niacin - 6 to 19 mg./day (USRDA 20 mg.); Selenium - 10 to 200 mcg./day; Zinc - 3 to 25 mg./day (USRDA 15 mg./day); Iron - 10 to 60 mg./day (USRDA 18 mg./day); Copper - .5 to 3.0 mg./day; Magnesium - 50-450 mg./day; Chromium - 10 to 200 mcg./day. The Handbook of Vitamins, Minerals and Hormones 2nd ed. Roman J. Kutsky, Ph.D., Van Nostrand Reinhold Company, New York (Applicant's Ex. 5), lists dose ranges as follows: Vitamin A - 1,300 to 50,000 I.U./day; Vitamin B_6 - .9 to 100 mg./day; Vitamin C - 45 to 1,000 mg./day; Vitamin E - 5 to 30 mg./day; Niacin - 9 to 1,000 mg./day (Niacinamide). The Merck Manual of Diagnosis and Therapy, 13th ed. Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 1977 (Applicant's Ex. 6), lists dose ranges as follows: Vitamin A - 1,400 to 6,000 I.U./day; Vitamin B_6 - .3 to 2.5 mg./day; Vitamin C - 35 to 80 mg./day (as ascorbic acid); Vitamin E - 4 to 15 I.U./day; Niacin - 5 to 20 mg./day; Zinc - 3

to 25 mg./day; Iron - 10 to 18 mg./day; and Cobalt - 20 to 30 mg./day (as cobaltous chloride).

As noted in The Right Dose, ". . . nutritional needs vary from person to person, depending on age, sex, health status and total diet." Given the large number of variables affecting the proper dosage of the aforementioned vitamins and trace elements, it is highly likely that even the aforementioned dose ranges would be inadequate to treat all patients under all conditions. The aforementioned reference materials demonstrate, however, that adequate dose ranges are readily available to even the layman. Additionally, it is clear that it would be obvious to one of ordinary skill in the art to deliver particular patients specific dosages based upon the patient's age, sex, health status, total diet, etc.

The Examiner argues that Dr. Kreamer is claiming a synergistic effect which is not clearly demonstrated on the record. The Examiner also states that there is no clear teaching of the proportions needed to effect the synergistic activity. In the previously submitted Declaration under 37 C.F.R. §1.132 of Larry H. Hollier, M.D. (Applicant's Ex. 7), Dr. Hollier declared that multi-vitamins are the most typical type of over-the-counter vitamin supplement taken by the general public. Furthermore, as shown by the attached and previously submitted labels from the most widely sold multi-vitamins (Applicant's Ex. 8), the dose ranges for the claimed vitamins and trace elements are generally equal to 100 percent or less of the United States recommended daily allowance and are no more than twice the United States

recommended daily allowance. As noted in the Declaration of Dr. Hollier, the data from the previously cited University of California study (Applicant's Ex. 1) shows that the actual effect of weekly administration of aspirin in combination with vitamins is a reduction in the risk of total deaths, cardiovascular deaths, myocardial infarctions, ischemic heart diseases, and other heart diseases beyond the anticipated additive effect of weekly administrations of aspirin in combination with vitamins.

Accordingly, since multi-vitamins are the most typical type of over-the-counter vitamin supplement taken by the general public, and since it is unlikely that subjects of the University of California study would exceed the suggested dosages labeled on the multi-vitamin and aspirin packages, it is highly likely that the known and recommended dosages of aspirin and multi-vitamins, when used in combination with one another, produce the unanticipated synergistic effects claimed by Dr. Kreamer.

> A decision on the issue of enablement requires determination of whether a person skilled in the pertinent art, using the knowledge available to such a person and the disclosure in the patent document, could make and use the invention without undue experimentation. It is not fatal if some experimentation is needed, for the patent document is not intended to be a production specification.

Northern Telecom, Inc. v. Data Point Corp., 15 U.S.P.Q.2d 1321, 1329 (Fed. Cir. 1990).

The issue of whether a disclosure requires undue experimentation to reap the benefits of an invention must be decided on a case-by-case basis. Ex parte Obukowicz, 27 U.S.P.Q.2d 1063 (Bd. Pat. App. Int. 1992). Dr. Kreamer

is not required to explain every detail of his invention since his specification is written to those skilled in the art. In re Howarth, 210 U.S.P.Q. 689, 691 (CCPA 1981). It is clear from Dr. Kreamer's specification and the level of skill in the art that undue experimentation would not be required to practice Dr. Kreamer's invention. Given the description in Dr. Kreamer's disclosure and the level of skill in the art, any physician of normal ability would be able to implement the claimed invention. Accordingly, Dr. Kreamer's disclosure meets the enablement requirement of §112. DeGeorge v. Bernier, 226 U.S.P.Q. 758, 762-63 (Fed. Cir. 1985). Dr. Kreamer should not be deprived of the fruit of his labor by too technical a reading of the requirement that his invention must be adequately described to indicate its true scope and content. Lever Bros. Co. v. Proctor & Gamble Mfg. Co., 60 U.S.P.Q. 76, 82 (4th Cir. 1943).

Accordingly, while Dr. Kreamer has not given specific dose ranges, it is respectfully submitted that his disclosure and claims are enabling under 35 U.S.C. §112. The practitioner of ordinary skill in the art would be readily able to provide a particular patient with the synergistic benefits of Dr. Kreamer's invention from the teaching in Dr. Kreamer's specification.

ISSUE 2:
The Rejection Of Claims 11-26 Under 36 U.S.C. §112

The Examiner argues that claims 11-26 fail to recite the active agents or the ratio of active agents which will yield the synergistic effect. The

Examiner states that the Declaration indicates that a combination of vitamins and minerals are used and not a single vitamin or mineral as claimed in claims 13-23. The Examiner argues that the claims are not commensurate with the scope of the data and that the data does not teach that each of the medicaments are effective individually or in a prescribed combination.

Dr. Kreamer agrees with the Examiner that the data from the University of California study does not specify dosages for each individual vitamin, trace element, and aspirin use (Applicant's Ex. 1). Because the data was collected and correlated by a third party unrelated to Dr. Kreamer, the data was not collected in the manner which would most effectively support Dr. Kreamer's claimed invention. Instead, the study was specifically drawn toward the relationship between aspirin use and chronic diseases (Applicant's Ex. 9). Dr. Kreamer contacted the party conducting the test and inquired as to whether information regarding vitamin use of the test subjects was recorded. The third party collected the data regarding aspirin usage and vitamin usage and provided this data to Dr. Kreamer. Although no dosages were provided to Dr. Kreamer from the third party conducting the study, it is unlikely that the resulting synergistic benefits shown in the study data were the result of patients exceeding the known dosages for the aspirin and multi-vitamins. It is more reasonable to conclude that the synergistic benefits of Dr. Kreamer's invention appear at known dosages of aspirin and the

vitamins and trace elements in question, which would be known to one of ordinary skill in the art from reading Dr. Kreamer's specification.

Although the University of California study supports the synergistic activity of Dr. Kreamer's claimed invention for typical dosages of aspirin and multi-vitamins, research has not yet been conducted to determine the outer limits of dose ranges sufficient to provide the noted synergistic effect. Studies sufficient to confirm the outer limits of these dose ranges would be prohibitably expensive for an independent inventor and would necessarily extend over several years. Accordingly, Dr. Kreamer has not yet conducted such a study and relies on the proven synergistic effect for known dosages and the level of skill in the art to provide synergistic results for a broad range of patients given the disclosure in Dr. Kreamer's specification.

> There are many situations in the practice of the arts in which specific directions are properly omitted from the claims of patents because greater definition is either impracticable or is unnecessary to inform the art, and would serve only unduly to limit the scope of the invention or to invite evasion by those who desire wrongfully to misappropriate the substance of the invention.

Lever Bros., 60 U.S.P.Q. at 82 (quoting Proctor & Gamble Mfg. Co. v. Refining, Inc., 57 U.S.P.Q. 505 at 511.

Given the diverse medical histories of patients, it would be impossible to define the outer limits of dose ranges for the claimed invention without extensive studies extending over several years. It would undermine the foundations of the Patent Act to deny an inventor the fruits of his lifesaving invention simply because the inventor has chosen to rely on dosages well-

known in the art. Furthermore, even these well-known dosages are subject to manipulation based upon a patient's condition. Dr. Kreamer should not be required to conduct extensive studies and limit his patent to narrowly defined dose ranges when both his disclosure and claims fully meet the requirements of 35 U.S.C. §112. .

ISSUE 3:
The Rejection Of Claims 11-26 Under 35 U.S.C. §103
As Being Unpatentable Over Igarashi, et al., Fratzer, and Frisbee

The Examiner states that Igarashi, et al. and Fratzer each teach in their abstracts the use of vitamin E to treat a vascular disease, and that Frisbee discloses at column 1, lines 25-30 that aspirin can be used to treat vascular diseases. The Examiner states that the combination of ingredients of known character, where the results obtained are no more than additive of the individual character, will not be patentable.

Judge Learned Hand noted over half a century ago that:

> [A]ll compositions are made of the same substances, retaining their fixed chemical properties. But the elements are capable of an infinity of permutations, and the selection of that group which proves serviceable to a given need may require a high degree of originality. It is that act of selection which is the single 'invention' and it must be beyond the capacity of commonplace imagination.

B.G. Corp. v. Walter Kidde & Co., 26 U.S.P.Q. 288 (2d Cir. 1935).

Dr. Kreamer admits that Igarashi, et al. teaches therapeutically treating hypertension with a vitamin E derivative, that Fratzer teaches normalizing blood coagulation through the use of vitamin E, and that Frisbee teaches treatment of vascular occlusive disease through the controlled release

of aspirin. Dr. Kreamer is fully aware of the numerous articles in the literature which discuss the effects of aspirin and vitamins on the human vascular system. The Examiner, however, has not cited a single reference, and Dr. Kreamer is aware of no such reference, which teaches or even suggests the inventive combination of aspirin with one or more of the claimed vitamins, or trace elements, or the unexpectedly synergistic effects of such a combination. In view of the great amount of literature, it is respectfully asserted that the nonobviousness of the claimed invention is supported, not defeated, by such references. Even if the Examiner had presented art which taught that each of the substances of the claimed compositions is individually taught in the art for reducing arteriosclerotic plaque formation, the presently claimed invention would not be obvious. To use the substances in combination for their known functions, many of which are antagonistic, cannot be regarded as obvious absent some teaching, suggestion or incentive supporting the combination. In re Geiger, 2 U.S.P.Q.2d 1276 (Fed. Cir. 1987). None of the cited references teaches nor suggests the combination of aspirin with the claimed vitamins or trace elements.

This case is similar to the facts presented in Ex parte Rubin, 5 U.S.P.Q.2d 1461 (Bd. Pat. App. & Int. 1987). The Rubin case dealt with a §103 rejection of a composition of interferon and tyrosinase inhibitor. Interferon was known to cause an increase in serum tyrosinase. The claimed combination increased the effectiveness of interferon by combining it with tyrosinase inhibitor. The claimed combination was not made obvious by the

references which taught the effectiveness of each component individually because there was no teaching, express or implied, of the combination. Similarly here, aspirin is known to inhibit the healing of arterial wounds by the irreversible blocking of platelet prostaglandin function, whereas vitamin A increases the rate at which reparative collagen accumulates at the wound. The combination of aspirin and vitamin A to enhance the effectiveness of aspirin alone is claimed, just as the combination of interferon and tyrosinase inhibitor was claimed in the Rubin case. The specific combination of aspirin and vitamin A is presented as exemplary only; each of the claimed combinations falls within the general rule of Rubin. The synergistic effect of the claimed combinations over the individual substances supports patentability.

Far from suggesting or teaching Dr. Kreamer's claimed combination, the relevant art teaches away from such a combination. Support for this teaching away is found in the aforementioned University of Southern California study on the effects of aspirin and the incidence of cardiovascular diseases (Applicant's Ex. 1). The study was undertaken by Dr. Annlia Paganini-Hill, et al. The study focused on the relationship between aspirin use and chronic diseases. Among the results of the study was the finding that the daily use of aspirin alone actually increased the risk of ischemic heart disease.

Dr. Kreamer contacted the conductor of the study, Dr. Paganini-Hill, and inquired as to whether her study recorded vitamin usage of the test

subjects. Dr. Paganini-Hill responded in the affirmative and provided Dr. Kreamer with the attached data, which has been previously submitted to the Examiner (Applicant's Ex. 1). As can be seen by the data, control subjects taking neither aspirin nor vitamins were given a relative risk rating of 1.00. For subjects taking vitamins and no aspirin, the relative risk for all deaths was 1.02, or .02 greater than those control subjects taking neither aspirin nor vitamins. For subjects taking aspirin and no vitamin, the relative risk for all deaths was found to be 1.00, no greater or worse than the control subjects. Accordingly, the anticipated additive effect of aspirin and vitamin use would be a .02 increase plus a .00 increase, or a .02 increase overall. However, as shown in the data, the actual use of aspirin and vitamins by test subjects showed a decrease in relative risk from 1.00 for the control subjects to .91 for subjects taking both aspirin and vitamins, a marked decrease of .09 in relative risk. This decrease of .09 in relative risk for all deaths stands in stark contrast to the anticipated additive effect of a .02 increase in relative risks for all deaths based upon the data for aspirin use alone and vitamin use alone. Similar results are shown for total cardiovascular deaths, acute myocardial infarction, ischemic heart disease, and other heart disease, with the results as follows:

The anticipated additive effect of aspirin and vitamin use on total cardiovascular deaths is a decrease of .08 for vitamin use and an increase of .09 for aspirin use, or a combined anticipated additive effect of a .01 increase in relative risk for total cardiovascular deaths.

The actual effect on total cardiovascular deaths through the administration of aspirin and vitamins is a .41 decrease in relative risk.

The anticipated additive effect of aspirin and vitamin use on acute myocardial infarction is a decrease of .14 for vitamin use and a decrease of .12 for aspirin use, or a combined anticipated additive effect of a .26 decrease in relative risk for acute myocardial infarction.

The actual effect on acute myocardial infarction through the administration of aspirin and vitamins is a .42 decrease in relative risk.

The anticipated additive effect of aspirin and vitamin use on ischemic heart disease is a decrease of .05 for vitamin use an increase of .40 for aspirin use, or a combined anticipated additive effect of a .35 increase of .35 in relative risk for ischemic heart disease.

The actual effect on ischemic heart disease through the administration of aspirin and vitamins is a .54 decrease in relative risk.

The anticipated additive effect of aspirin and vitamin use on other heart disease is an increase of .46 for vitamin use an increase of .52 for aspirin use, or a combined anticipated additive effect of a .98 increase in relative risk for other heart disease.

The actual effect on other heart disease through the administration of aspirin and vitamins is a .03 increase in relative risk.

Dr. Kreamer does admit, that while the decrease in relative risk for stroke is extremely significant for the combination of aspirin and vitamin and far more beneficial than either aspirin or vitamin alone, the effect is not more

than the anticipated additive effect of the combination of aspirin and vitamins. It is apparent, however, that overall the actual combination of vitamin and aspirin together is markedly more beneficial than aspirin alone, vitamin alone, or the anticipated additive combination of the two.

From the aforementioned data, it can be seen that the anticipated results of combining aspirin with vitamins would actually be an increase in the risk of all deaths, total cardiovascular deaths, and ischemic heart disease. From a review of the data relating to the administration of aspirin alone and the administration of vitamins alone, the data clearly teaches that the anticipated additive effect would be an increase in the relative risk of death over those patients taking neither aspirin nor vitamins. The art, therefore, clearly teaches away from administering such a combination to a patient. Given the anticipated result of combining aspirin with vitamins of an increase in the risk of death from total cardiovascular deaths, ischemic heart disease and all deaths together, the combination of aspirin with vitamins would be contraindicated based upon the data. It would simply not be obvious to one of ordinary skill in the art to try such an anticipatedly harmful combination on a human patient. Surprisingly, however, in direct contravention to the anticipated increase in relative risk of death, the administration of vitamins along with aspirin actually reduces the relative risk of death from all of the aforementioned conditions including all deaths. Accordingly, Dr. Kreamer's claimed combination produces a synergistic reduction in deaths in the face of an anticipated increase in deaths.

While not specifically drawn to the study of aspirin, multi-vitamins, and heart disease, Dr. Paganini-Hill's article explains the statistical significance of the data provided in the table and utilized by Dr. Kreamer to support the unanticipated beneficial aspects of his inventive method (Applicant's Ex. 9). As can be seen by Dr. Paganini-Hill's article, the study data not only shows unanticipatedly beneficial results, but results which are statistically significant within confidence levels accepted in the industry. Accordingly, Dr. Kreamer respectfully submits that not only is the claimed method not harmful, as would be anticipated from the submitted data on vitamin usage alone and aspirin usage alone, but that the claimed method is actually unanticipatedly beneficial and, therefore, would be nonobvious to one of ordinary skill in the art.

Based upon the foregoing, it is respectfully requested that the Examiner's decision be reversed.

Appendix (37 C.F.R. §1.192(c)(7))

Dr. Kreamer's Amendment of October 13, 1994, was not entered. The Examiner stated that the amendments raised new issues that would require further consideration and/or search, and that they presented additional claims without canceling a corresponding number of finally rejected claims. Accordingly, the claims which follow do not include Dr. Kreamer's amendments of October 13, 1994.

11. A method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans through the reduction of cholesterol incorporation into the endothelium, comprising:

(a) blocking prostaglandin function in platelets through the oral administration of aspirin to reduce the ability of platelets to go to the site of endothelial damage and recruit other cells to assist in thrombosis; and

(b) reducing the migration of cholesterol into the endothelium through the oral administration of medicament.

12. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, wherein said medicament is at least one vitamin selected from the group consisting of vitamin A, vitamin B_6, vitamin C, vitamin E, and niacin.

13. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising increasing prostaglandin syntheses through the oral administration of at least one vitamin selected from the group consisting of vitamin C, vitamin E, and niacin as said medicament.

14. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing serum cholesterol through the oral administration of at least one

vitamin selected from the group consisting of vitamin B_6, vitamin C, vitamin E, and niacin as said medicament.

15. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising speeding the healing of the endothelial damage through the oral administration of vitamin A as said medicament.

16. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing standard clot formation through the oral administration of at least one vitamin selected from the group consisting of vitamin A and vitamin E as said medicament.

17. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing immune-induced lesions through the oral administration of vitamin E as said medicament.

18. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans through the oral administration of vitamin E as said medicament.

19. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, wherein said medicament is at least one trace element selected from the group consisting of chromium, selenium, zinc, iron, copper, cobalt, and magnesium as said medicament.

20. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing serum cholesterol through the oral administration of at least one trace element selected from the group consisting of chromium, copper, magnesium, selenium, and zinc, as said medicament.

21. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising increasing platelet activity through the oral administration of at least one trace element selected from the group consisting of magnesium and selenium as said medicament.

22. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising increasing prostaglandin synthesis through the oral administration of selenium as said medicament.

23. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising speeding the healing of endothelial damage through the oral administration of at least one trace element selected from the group consisting of copper and magnesium as said medicament.

24. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing standard clot formation through the oral administration of selenium as said medicament.

25. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing immune-induced lesions through the oral administration of at least one trace element selected from the group consisting of copper and selenium as said medicament.

26. The method for reducing arteriosclerotic plaque formation at sites of endothelial damage in humans of claim 11, further comprising decreasing peroxidation through the oral administration of selenium as said medicament.

Respectfully submitted,

Date: January 18, 1995

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ATTORNEYS FOR DR. KREAMER

TABLE 3

Aspirin and Vitamin Use	All Deaths		Total Cardiovascular Deaths		Myocardial Infarction		Ischemic Heart Disease		Other Heart Disease		Stroke	
	No.	RR	No.	RR	No.	RR	No.	RR	No.	RR	No.	RR
Men:												
Aspirin- Vitamin-	320	1.00	161	1.00	54	1.00	45	1.00	29	1.00	29	1.0
Aspirin- Vitamin+	450	1.02	203	0.92	64	0.86	58	0.95	58	1.46	26	0.6
Aspirin+ Vitamin-	45	1.00	24	1.09	7	0.88	8	1.40	6	1.52	2	0.5
Aspirin+ Vitamin+	91	0.91	29	0.59	10	0.58	6	0.46	9	1.03	3	0.3
Aspirin++ Vitamin-	65	1.35	27	1.09	7	0.91	10	1.37	7	1.57	7	1.5
Aspirin++ Vitamin+	112	1.01	51	0.90	11	0.61	13	0.78	13	1.26	12	1.1
Vitamin+		0.96		0.85		0.80		0.78		1.07		0.6
Women:												
Aspirin- Vitamin-	326	1.00	166	1.00	46	1.00	50	1.00	44	1.00	33	1.0
Aspirin- Vitamin+	561	0.84	263	0.78	72	0.77	69	0.67	57	0.63	67	1.0
Aspirin+ Vitamin-	47	0.82	26	0.91	4	0.49	9	1.06	6	0.81	4	0.6
Aspirin+ Vitamin+	119	0.74	58	0.73	14	0.63	20	0.86	12	0.59	7	0.4
Aspirin++ Vitamin-	77	1.36	45	1.48	10	1.25	14	1.50	12	1.41	9	1.5
Aspirin++ Vitamin+	163	1.08	86	1.10	24	1.13	23	0.97	24	1.14	14	0.9
Vitamin+		0.85		0.78		0.83		0.70		0.68		0.8

Aspirin use: - = none; + = weekly or less often; ++ = daily
Vitamin use: - = no; + = yes

Clinical trials evaluating platelet-modifying drugs in patients with atherosclerotic cardiovascular disease and thrombosis

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ABSTRACT Aspirin has been convincingly shown to reduce (1) stroke and death in men with transient ischemic attacks (it may possibly be beneficial to women also), (2) myocardial infarction and death in patients with unstable angina, (3) thromboembolic complications associated with artificial heart valves in patients receiving oral anticoagulants (although gastrointestinal bleeding is prohibitive with this combination), and (4) thrombotic occlusion of silicone rubber arteriovenous cannulae in uremic patients undergoing hemodialysis. In addition, aspirin may possibly decrease occlusion of saphenous vein aortocoronary grafts and venous thrombosis in men after hip replacement, although these reports require confirmation. Aspirin is ineffective in the secondary prevention of stroke and has unproven benefit in the secondary prevention of myocardial infarction. Dipyridamole in combination with oral anticoagulation decreases the thromboembolic complications associated with mechanical heart valves. The combination of aspirin and dipyridamole prevents both early and late occlusion of saphenous vein aortocoronary bypass grafts and protects renal function in patients with membranoproliferative glomerulonephritis. The relative importance of combining aspirin and dipyridamole compared with either agent used singly remains to be established. Sulfinpyrazone reduces the thrombotic occlusion of arteriovenous cannulae and early occlusion of saphenous vein aortocoronary grafts. The reported benefit in the secondary prevention of myocardial infarction is controversial.
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ALTHOUGH antiplatelet drugs reduce thrombotic and thromboembolic complications in a number of arterial vascular disorders and in patients with prosthetic cardiovascular devices, the indications for the clinical use of agents that modify platelet function in the management of patients with arterial cardiovascular disease are not entirely clear despite extensive basic, experimental animal, and clinical studies.[1-3] In general, the negative clinical trials have been uninformative because of uncertainty with respect to the role of platelets in the pathogenesis of the various clinical outcome events, because of inadequately defined mechanisms of drug actions and uncertainty regarding the effective drug doses in the trials, and because of the lack of suitable pharmacologic agents for definitive clinical studies and the inconclusive results of many completed trials.

In this article, the current status of antiplatelet therapy in clinical practice is reviewed with particular emphasis on the results of well-designed, controlled clinical trials in patients with vascular thrombotic syndromes. The quality of the evidence from the reported trials has been assessed on the basis of trial design, execution, and data analysis. Basic principles and issues considered to be of fundamental importance in the evaluation of clinical trials include specific inclusion-exclusion criteria, randomization, stratification for important prognostic factors, double-blind administration of therapy, assessment of drug compliance and contamination, appropriate and well-defined outcome events, assessment of adverse effects, and the use of sound statistical methods.[1, 2]

Ideally, a clinically useful, platelet-modifying drug should be nontoxic and orally effective, have sustained action, and have good antithrombotic potency without excessive risk of abnormal bleeding. None of the currently available clinical agents satisfies all these requirements. Aspirin, sulfinpyrazone, dipyridamole, suloctidil, and ticlopidine have been the agents evaluated in clinical trials to date.

In the absence of established mechanisms of antithrombotic drug action, dose regimens must be determined with the use of objective assays and thrombotic end points rather than the use of biochemical mea-

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surements in vitro of less certain interpretation. Many agents with more defined pharmacologic actions have been synthesized for evaluation and are presently at varying stages of development.

Thrombus formation. Thrombogenesis involves (1) exposure of a thrombogenic surface to blood; (2) platelet adherence, aggregation, and release, and (3) thrombin generation and fibrin formation. Thrombus dissolution requires generation of plasmin and fibrinolysis. However, the blood vessels, platelets, clotting factors, and fibrinolytic components and inhibitors interact physiologically and pathologically in a variety of complex ways to promote and limit thrombus formation.

The structure, size, and localization of thrombi are profoundly influenced by blood flow. In general, exposure of a thrombogenic surface to flowing blood at high shear induces the formation of thrombus composed predominantly of platelets (white thrombus), whereas a thrombus that forms in a vein with stagnant blood flow is rich in fibrin and red blood cells (red thrombus). Kinetic studies using radioactively labeled platelets and fibrinogen document these distinctions between arterial and venous thrombi.[4] Arterial thrombosis is characterized by selective platelet consumption that can be interrupted by some inhibitors of platelet function but not by heparin. On the other hand, venous thrombosis involves combined and equivalent consumption of both platelets and fibrinogen. This process is blocked by anticoagulation but not by drugs that inhibit platelet function. However, since each clinical vascular syndrome probably involves a unique site and pathogenetic mechanism, the effects of the different classes of antithrombotic agents and specific drugs within each class may be quite different in the various clinical settings.

Pathophysiology of arterial insufficiency syndromes

Transient ischemic attacks. Evidence from clinical and pathologic studies suggests that platelets may participate in the pathogenesis of ischemic syndromes involving the cerebral circulation. For example, patients with transient ischemic attacks (TIAs) frequently have ulcerated atherosclerotic lesions in the extracranial portion of the basilar or internal carotid arteries. TIAs may be the result of microembolization from thrombi forming on such lesions.[5] Moreover, embolic material has been observed during its passage through the retinal arterioles while attacks of amaurosis fugax are in progress.[6] Some of these microemboli are composed of platelets. Alternatively, vasospasm at stenotic sites of diseased vessels may also be responsible for transient ischemia. Indeed, it should be noted that transient cerebral ischemia may be due to a variety of causes other than thromboembolism and vasospasm, including hemodynamic changes, cardiac arrhythmias, prolapse of the mitral valve, atrial myxomas, nonarteriosclerotic vasculopathies, embolism of atheroma, and cervical spondylosis.[7] Hence, any large clinical series contains patients with cerebral ischemia of varying causes. The relative importance of platelet aggregates as a cause of TIA is unknown.

Acute myocardial infarction. Recent angiographic studies confirm that acute transmural myocardial infarction is generally associated with thrombotic occlusion of the subtending diseased coronary artery.[8, 9] An occlusive thrombus can be demonstrated by coronary arteriography in at least 90% of patients with transmural infarction if angiography is performed within the first several hours after the onset of symptoms. Although occlusion of a stenotic coronary artery may theoretically occur in a number of different ways, the high frequency of reperfusion achieved by fibrinolytic therapy suggests that denudation of an atherosclerotic plaque and consequent thrombosis is the typical course. Occlusion by intimal hemorrhage or thrombosis induced by discharge of the contents of the plaque is probably uncommon.

Coronary vasospasm may also be important in some forms of myocardial infarction. In that respect, Maseri et al.[10] showed that spontaneous coronary artery spasm precipitated angina and that repeated episodes could lead to myocardial infarction in some patients.

The interaction of circulating blood elements and some of their secreted products with the vessel wall may be important in the development of coronary vasospasm.[11] Thromboxane A_2 is a potent labile vasoconstrictor released during myocardial ischemia, perhaps through the activation of platelets, leukocytes, or atherosclerotic vascular wall components.[12] Serotonin increases coronary vascular tone, whereas locally released adenosine and adenosine nucleotides induce vasodilatation. ADP- and ATP-mediated smooth muscle relaxation is dependent on an intact and functional endothelium, whereas adenosine and AMP produce a direct effect on smooth muscle cells. Adenosine also promotes relaxation of vascular smooth muscle by inhibiting the release of norepinephrine from sympathetic nerve terminals. Of those substances, thromboxane A_2, which induces vasoconstriction, may be the most significant during thrombus deposition.

In addition, leukocytes and a diseased vascular wall may directly or indirectly modulate coronary artery tone in association with myocardial ischemia, either alone or in concert with thrombus formation.[11] Toxic oxygen products[13] or released neutral proteases from

granulocytes[14] may contribute to endothelial cell detachment and thus to an increase in the amount of exposed thrombogenic surfaces, thereby extending the amount of thrombus that forms. Leukotrienes cause potent dose-dependent coronary artery vasoconstriction and decreased myocardial contractility in isolated animal preparations. Although their role in coronary artery spasm has not been defined in vivo, it is possible that they may contribute to sustained arterial spasm. Thus, endothelial denudation and vascular spasm may precede or follow acute thrombosis of atherosclerotic coronary arteries and contribute to an extension of infarction.

Sudden death. The immediate factor or factors that precipitate arrhythmias in victims of out-of-hospital ventricular fibrillation remain largely undefined.[2] Traditional risk factors associated with coronary atherosclerosis (smoking, hypertension, and hyperlipidemia) are also associated with sudden death. Postmortem studies of people who died suddenly of cardiac causes do not show pathologic thrombosis of coronary arteries. However, such patients do have advanced disease with significant stenoses in several vessels, and the presence of platelet aggregates has been documented after death.

In some experimental studies ADP-activated platelets provoked terminal arrhythmias, possibly by producing myocardial ischemia through thromboxane A_2-induced coronary artery spasm or occlusion of myocardial microcirculation by microemboli. In that context, inhibition of thromboxane A_2 synthesis reduces the frequency of arrhythmias or ventricular fibrillation in several animal preparations. In other experimental studies, platelet emboli in the microcirculation cause ventricular fibrillation and sudden death. Those results suggest that platelet microemboli from coronary mural thrombi may obstruct the coronary microcirculation and precipitate arrhythmias. The relationship between and the relative importance of platelet plugs and vasospasm with respect to other potential mechanisms in sudden cardiac death is unclear.

Unstable angina pectoris. Evidence in favor of a role for thrombosis in typical, variant, or unstable angina pectoris is not convincing.[15] However, recent studies in vivo measuring platelet function during exercise or cardiac pacing show changes in coronary sinus levels of thromboxane B_2, the stable metabolite of thromboxane A_2.[12] Elevation of thromboxane B_2 in the coronary sinus correlated with the time course of increased lactate production, suggesting a relationship between formation of thromboxane A_2 and myocardial ischemia.

Patients with unstable angina and recent chest pain also have higher thromboxane B_2 levels in the coronary sinus than in the ascending aorta, which suggests that recent episodes of angina in such patients are associated with local release of thromboxane A_2.[12, 16] The capacity of aspirin to reduce the frequency of myocardial infarction in patients with unstable angina[17] is consistent with the concept that aspirin might be acting through a blockade of thromboxane A_2 production. Thus, the results of that trial are consistent with the notion that thromboxane A_2 may be involved in unstable angina. This same mechanism may also underly the benefit of aspirin in patients with TIAs.[18, 19] However, it should be pointed out that there is difficulty in using measurements of thromboxane B_2 in biological fluids as an index of thromboxane A_2 generation in vivo. In many studies the levels of plasma thromboxane B_2 reported are clearly artifactual. With more care, and with the use of special cannulae, lower baseline levels of plasma thromboxane B_2 have been demonstrated, and even these reduced values may exceed true levels. Indeed, samples withdrawn through long cannulae may never give precise results. Therefore, the evidence from studies using such measurements are questionable.

A role for platelets in variant angina (characterized by pain at rest, ST segment elevation, and angiographic evidence of coronary artery spasm) is of great interest. It has been suggested that platelet activation, aggregation, and release of thromboxane A_2 might trigger episodes of spasm. Elevations in thromboxane B_2 and increased levels of circulating platelet aggregates have been described during episodes of pain at rest and ST segment elevation in patients with variant angina. However, double-blind crossover studies using inhibitors of platelet thromboxane synthesis in those patients show no reduction in angina pain or electrocardiographic changes, despite the blockade of thromboxane A_2 release from platelets.[16]

Membranoproliferative glomerulonephritis. A role for platelets in the pathogenesis of the primary glomerular disease membranoproliferative glomerulonephritis was implied by the findings that selective platelet consumption is found in this disorder and that platelet consumption is interrupted by administration of combination aspirin-dipyridamole.[20] Moreover, neutrophil-macrophage chemataxis and mesangial cell proliferation in the glomeruli might be mediated by the platelet-derived growth factor released during platelet activation.[21, 22] Thus, both the inflammatory and proliferative aspects of the pathologic picture may be platelet dependent. In addition, the generation of thromboxane A_2 may act as an important nonimmune mediator

of glomerular injury by inducing vasospasm, platelet aggregation, and leukocyte adhesiveness.[23]

Saphenous vein coronary artery bypass grafts. Coronary artery bypass grafting (CAGB) with the autogenous saphenous vein is performed for patients with angina due to coronary artery disease. Over 90% of patients can be expected to have improvement or relief of symptoms of angina for at least 1 year after surgery. Since the proposed mechanism for this symptomatic improvement is increased blood flow to ischemic myocardium, the efficacy of CABG is a function of the vein graft patency rate. Overall, graft patency rates are reported to be 90% at 2 weeks and 65% to 85% at 1 year. The major factors contributing to vein graft occlusion are thrombosis and intimal proliferation in the graft itself. It is generally believed that early vein graft occlusion occurs secondary to thrombosis and that later graft occlusion is related to intimal proliferation of the vein wall. Thrombotic occlusion predominantly involves platelets due to local vascular injury and high-shear hemodynamic factors.[15] The process of intimal proliferation is progressive and is postulated to result from exposure of the vein grafts to arterial pressure and damage to the endothelium of the vein, followed by platelet-dependent intimal proliferation of smooth muscle cells.[21] These processes ultimately result in platelet deposition and occlusion of grafts.

Pathophysiology of thrombus formation with artificial surfaces

Prosthetic heart valves. Whereas normal endothelium is nonreactive with circulating blood, prosthetic surfaces generally activate platelets and coagulation factors to initiate thrombus formation. In association with cardiac valve prostheses there is formation of both fibrin ("red") thrombus and platelet ("white") thrombus. Red thrombus forms secondary to stasis, as a consequence of atrial dilatation, atrial fibrillation, and the interrupted flow occurring during the cardiac cycle. Platelet thrombi form on the prosthetic valve surface because the artificial surface is exposed to blood under high-shear conditions. ^{111}In-platelet deposition has been directly observed on the prosthetic surfaces as well as on the immediately adjacent damaged vascular surfaces. Platelet survival measurements in patients with prosthetic cardiovascular devices have been shown to be of value in identifying patients at risk and in predicting the effects of therapy.[24, 25] With respect to mechanical prosthetic heart valves, this thromboembolic risk is related at least in part to the exposed surface area. Thus, as the prosthetic surface area increases, the thromboembolic risk rises in parallel and the platelet survival time shortens proportionately. In patients with homograft cardiac valves, no detectable shortening of platelet survival has been found and a marked reduction in the frequency of thromboembolic events is observed. Platelet survival time has also been useful in evaluating the effects of various pharmacologic agents. When dipyridamole is administered in doses of 100 mg four times daily to patients with mitral prosthetic heart valves or silicone rubber arteriovenous cannulae the platelet survival time is normalized, implying an interruption of the thrombotic process. Sulfinpyrazone has also been reported to normalize platelet survival at doses of 200 mg four times per day.[25]

Arteriovenous silicone rubber cannulae. In patients with chronic uremia undergoing hemodialysis by way of arteriovenous silicone rubber cannulae, shunt occlusion by thrombus is sufficiently frequent that the antithrombotic efficacy of drugs may be assessed in terms of reduction in the number of thrombotic occlusive events and in terms of patients developing thrombi. Typically, platelet thrombus forms at the vein-shunt junction[26] because of the intimal thickening that forms at the site, perhaps mediated by platelet-derived growth factor.[21]

However, the use of this clinical model of thrombosis for the assessment of antithrombotic agents is complicated by the presence of underlying platelet dysfunction in patients receiving hemodialysis (in contrast to uremic patients being treated by peritoneal dialysis).[27] Thus, antithrombotic efficacy of an agent at a given dose in this model may not be effective in patients without the platelet dysfunction caused by hemodialysis.

Clinical trials of platelet-modifying agents

TIAs. The evaluation of antiplatelet therapy in cerebrovascular ischemia is difficult because there are problems with accumulating sufficient numbers of patients to allow statistically significant differences to be observed. This difficulty arises from the limited availability of suitable patients and from the low frequency of the two clinically important outcome events, stroke and death. Therefore, long-term follow-up observation and large-scale multicenter trials are necessary.

Approximately 25% to 40% of patients with TIAs will develop cerebral infarction within 5 years of the initial event.[7] The period of greatest risk is the first year after the onset of TIAs, i.e., one-half of the patients develop cerebral infarction within the first year and the majority occur within the first 30 days. Recurrent TIAs carry a particularly poor prognosis for the development of cerebral infarction. After the first year, the rate of stroke occurrence is approximately 5% per year, which is five times as great as the stroke rate

in the general population of similar age. Most patients who have TIAs die of vascular causes, one-third of them with stroke and two-thirds with other vascular diseases, primarily myocardial infarction.

Acheson et al.[28] reported the results of a randomized double-blind trial assessing the effect of dipyridamole in 153 patients with TIAs or stroke. Patients were randomized to receive either placebo or dipyridamole in doses of 400 mg/day. Fourteen months later the patients remaining in the study were treated with 800 mg/day dipyridamole and followed for an additional 11 months. No significant difference was found between the control and dipyridamole-treated patients in the frequency of recurrent TIAs, ischemic stroke, or death, although the relatively small number of patients does not exclude the possibility of a true benefit.

The Aspirin in Transient Ischemic Attacks Study[18] was designed to test, in a double-blind manner, the effectiveness of aspirin in the prophylaxis of cerebrovascular ischemic events. Only patients with carotid system TIAs were admitted to the study. Subjects received either 650 mg aspirin twice a day or appropriate placebo. Analysis of the first 6 months of follow-up showed a statistically significant difference in favor of aspirin when death, cerebral or retinal infarction, and the occurrence of TIAs were grouped together as end points. The primary outcome was defined as stroke-free survival with reduced TIAs during the first 6 months of follow-up. Among the 178 patients studied, favorable outcomes were reported in 81% of the aspirin-treated group compared with 56% in the placebo control group ($p < .01$). When stroke or death was considered as a composite outcome event, the beneficial effects of aspirin were confined to men (47% vs 0% reduction for women). When the occurrence of TIAs was included as part of the outcome and results were classified as favorable or unfavorable, there was no sex difference in the response to aspirin.

Fields et al.[29] carried out a second study to assess the effect of aspirin in 125 patients who had operations of the carotid artery after episodes of TIA. These patients were randomly assigned to aspirin, 1200 mg/day, or placebo. Favorable outcomes, defined as stroke-free survival with reduced TIAs during the first 6 months, were noted in 89% of the aspirin-treated group and 76% of the placebo-treated group. Aspirin had no significant effect either on overall mortality or on the frequency of cerebral or retinal infarctions. When deaths that were not related to stroke were eliminated from this analysis, there was an observed reduction in the frequency of fatal or nonfatal cerebral and retinal infarctions in the aspirin-treated group compared with the placebo-treated group. However, because of the small number of patients and the short period of follow-up, these results by themselves do not establish convincingly that aspirin was effective in preventing cerebral infarction.

The Canadian Cooperative Study Group was a double-blind, randomized, multicenter trial to assess the relative efficiency of aspirin and sulfinpyrazone, singly and in combination, in the reduction of continuing TIAs, stroke, and death.[19] Five hundred eighty-five patients (after 64 exclusions) who had suffered one or more cerebral or retinal ischemic attacks within 3 months before entry were followed in a randomized clinical trial for an average of 26 months. About 30 of the patients were women. The four treatment regimens were placebo, aspirin (325 mg four times daily), sulfinpyrazine (200 mg four times daily), and aspirin plus sulfinpyrazone, at the same dosage. For the entire group, aspirin reduced the risk of continued TIAs, stroke, or death by 19% ($p < .05$). If only stroke or death was considered, aspirin reduced the risk by 31% ($p < .05$). There was no statistically significant reduction in these events attributable to sulfinpyrazone. A striking difference was found between male and female patients with respect to the therapeutic responses to aspirin ($p < .003$) in that there was no observed benefit among women in terms of stroke or death, but there was a risk reduction of 48% among men.

Guiraud-Chaumeil et al.[30] randomly allocated 440 patients presenting with TIAs to one of three treatment groups, all of which received dihydroergocornine (4.5 g daily); one group received nothing else, one group also received aspirin (900 mg daily) and the third group received aspirin (900 mg daily) plus dipyridamole (150 mg daily). The investigators in this single-center, unblinded trial concluded that there were no statistically significant differences in outcomes. However, while the actuarial curves for the outcomes of stroke or death were very similar for the aspirin and aspirin plus dipyridamole groups, they were both appreciably better than for the dihydroergocornine alone group.

Sorensen et al.[31] recently published the results of a trial involving 203 patients, 148 men and 55 women, who during the month before admission had experienced at least one reversible cerebral ischemic attack of less than 72 hours' duration. They were randomly assigned to treatment with either aspirin (1000 mg daily) or placebo and followed for an average of 25 months. The frequency of stroke or death was 21% in

the aspirin group and 17% in the placebo group. Moreover, the occurrence of TIAs during the treatment period was also not reduced by aspirin treatment.

There were fewer myocardial infarctions observed in the aspirin group (5.9%) than in the placebo group (13.7%); this difference, however, was also not statistically significant ($p = .10$). This negative study was not of sufficient size to exclude the possibility that aspirin treatment could truly be effective.

Bosser et al.[32] carried out a controlled cooperative trial in France to evaluate the effect of aspirin singly or in combination with dipyridamole in the secondary prevention of cerebral ischemic accidents. A total of 604 patients with atherothrombotic cerebral ischemic events (transient 16%; minor stroke 84%) referrable either to the carotid or to the vertebral-basilar circulation were entered into the double-blind randomized clinical trial to compare the effects of aspirin (1 g/day; 325 mg three times daily) or aspirin (1 g/day; 325 mg three times daily) in combination with dipyridamole (225 mg/day; 75 mg three times daily) and were followed for 3 years. Randomization produced comparable treatment groups; adherence to the protocol and drug compliance were good. However, side effects, particularly symptoms of peptic ulcer and hemorrhagic events were significantly more frequent ($p < .03$) in the two treatment groups receiving aspirin. At the end of the study, the number of fatal and nonfatal cerebral infarctions was 31 in the placebo group, 17 in the aspirin group, and 18 in the combination aspirin-dipyridamole group, corresponding to cumulative rates of 18% in the placebo group and 10.5% in each of the two active treatment groups. A difference at the 6% level was present between placebo and combination aspirin-dipyridamole and between placebo and aspirin. Clearly, no difference between aspirin and aspirin plus dipyridamole was observed. Among other diseases observed during the trial, the only significant difference concerned myocardial infarction, which was less frequent in the two treated groups ($p < .05$). Interestingly, subgroup analysis failed to show a significant sex difference in the efficacy of aspirin.

Recently, the American-Canadian Study Group has completed a multicenter randomized trial to determine if the combination of aspirin (1.3 g daily) and dipyridamole (300 mg daily) offered any benefit over aspirin (1.3 g daily) alone.[33] A total of 890 patients with a history of recent carotid territory TIAs was followed for an average of 25 months and the outcomes in the two groups were almost identical.

In an ongoing study of patients suffering TIAs in the United Kingdom,[34] the effect of different aspirin doses is being compared. In this study, 3000 patients with TIAs are randomly allocated to placebo, 300 mg aspirin daily, or 1.2 g aspirin daily. The results are expected to be reported in 1987.

The Ticlopidine-Aspirin Stroke Study is an ongoing multicenter study to evaluate the ischemic stroke prevention efficacy of ticlopidine compared with the active control drug aspirin.[35] Study patients have histories of one or more cerebral or retinal TIAs, one or more attacks of cerebral ischemia or reversible ischemic neurologic disease, and/or a minor stroke followed by a greater than 80% functional recovery within 3 months before trial entry (currently 82% have transient cerebral or retinal ischemia as the qualifying event). It is planned to enter 3000 patients over a 3 year recruitment period with a 2 to 5 year follow-up. Results are expected to be reported in 1988.

In conclusion, the results of several of the large multicenter TIA trials have many similarities with respect to the response to aspirin. There was substantial reduction in TIAs, but more importantly, a decrease in stroke and death, especially from vascular causes. The conclusions of two of these studies are convincing and consistent regarding reductions in mortality and incidence of stroke with 1 to 1.3 g daily treatment with aspirin. Although no definite interaction was noted between aspirin and sulfinpyrazone, the data do not exclude that possibility, and this consideration remains an open question. The observations concerning the difference in response according to sex was also similar among the studies in that reduction of death was predominantly in men. In all studies, aspirin was well tolerated. The complications, mostly gastrointestinal, were less frequent in the cerebrovascular ischemia studies than in the myocardial infarction studies, despite similar aspirin dosages.

However, these studies leave unanswered the question of the optimal aspirin dose and schedule. In view of the current interest in low-dose aspirin regimens (see below), there is a clear need for studies to define the dose response. The U.K. TIA study[34] will provide valuable information in this regard. Of additional concern is the question of how to provide effective therapy for symptomatic women. Finally, the results of these trials do not discriminate between platelet thrombi vs vasospasm as the underlying mechanism of TIAs since aspirin-inactivated cyclooxyenase would also block thromboxane A_2–mediated vasoconstriction.

In summary, aspirin has been convincingly shown to reduce stroke and death in men with TIAs and may

possibly be beneficial to women also. No benefit has been shown by combining aspirin with dipyridamole and there is probably no additional benefit of sulfinpyrazone. No benefit has been found for sulfinpyrazone, dipyridamole, or suloctidil alone in the prevention of stroke and death in patients with cerebrovascular disease. Results with ticlopidine are pending.

Secondary prevention of myocardial infarction. Clinical trials in coronary heart disease present major difficulties, primarily related to the large number of patients required to perform the studies in a reasonable time, since the frequency of the important outcome events, myocardial infarction and death, is low. If a primary prevention study were performed on randomly selected middle-aged subjects without evidence of overt coronary artery disease, and myocardial infarction or death from myocardial ischemia were used as outcome events, many thousand patient-years of study would be required to detect a clinically significant reduction in outcome, since the expected frequency of such outcomes in untreated patients is approximately 1% per year.[1, 2] If the study were restrictd to symptomatic patients with demonstrated coronary disease, the numbers required would still be considerable, since the expected frequency of myocardial reinfarction or death is about 5% per year, i.e., the study would need more than 2000 patient-years to detect statistically significance differences, assuming that the true effect of treatment would be to produce a 50% reduction in events. However, since it is likely that myocardial infarction and death result from multiple factors, some of which may not involve platelet mechanisms, and in view of the limited pharmacologic tools available, reductions as large as 50% should not be expected.

The development of a first episode of transmural infarction serves as a valid outcome of the failure of an antithrombotic regimen in primary prevention trials, and the occurrence of reinfarction is a valid outcome measure in secondary prevention studies. However, since patients with transmural infarcts may die suddenly and cannot be distinguished from patients with unheralded sudden death due to arrhythmias or pump failure, which would not be expected to respond to antithrombotic measures, even the "hardest" outcome of all, death, may not necessarily be an efficient measure of the efficacy of an antithrombotic regimen. Thus, the natural history of myocardial infarction is an important consideration in the design and interpretation of intervention trials.[2]

Each year in the United States alone there are about one million "heart attacks" — a term that includes both acute infarction and sudden cardiac death. Nearly 200,000 victims die before admission to the hospital and about another 200,000 die in the first month, most of them in the first 24 hr after the attack. Thus, the cumulative mortality during the first month is about 40%. Of the 600,000 patients who survive the first month, about 10% die during the following year. About two-thirds of these deaths occur in the first 6 months, and they are generally due to sudden death or recurrent infarction. For patients who survive this year, the reinfarction rate and death rate stabilizes at 3% to 5% per year. This rate is similar to that for patients with symptomatic coronary artery disease who have not sustained a myocardial infarction previously, i.e., patients with angina pectoris, but is significantly greater than for a randomly selected, age-matched population without overt coronary artery disease that has a 1% yearly incidence of those events.

The logistical problems of conducting a primary prevention trial are illustrated by the cooperative trial that tested the efficacy of clofibrate in reducing the incidence of myocardial infarction or sudden death.[36] Apart from the enormous cost involved in a trial requiring nearly 100,000 patient-years of follow-up, the design of a primary prevention trial to test the efficacy of antithrombotic drugs must also address the difficult question of drug safety. For example, one would clearly hesitate to subject 99% of a normal population to the hazards of anticoagulant therapy in order to prevent myocardial infarction or sudden death in a portion of the remaining 1%. Even with a relatively "benign" antithrombotic drug such as aspirin, the complications of therapy in a primary prevention trial may outweigh the potential benefits. The trial with clofibrate[36] illustrates this point, i.e., although nonfatal infarctions were reduced with this drug, there was actually a net increase in mortality due to an increased frequency of death from biliary and gastrointestinal disorders. From a public health perspective overall mortality is clearly an important outcome event to be considered in all trial protocols.

Since prior myocardial infarction is a potent risk factor in identifying a high-risk group of patients in a general population, secondary prevention trials are more practical. Although the disease process in these patients is not advanced, they have had a clear-cut marker event for thrombosis. Because of the increased frequency of reinfarction or death in these patients, the number of patient-years required to detect a significant drug effect is substantially less than in corresponding primary prevention trials. However, even within the relatively well-defined study population of survivors of infarction, there are important points that must be

considered in designing and interpreting intervention trials. First, the prognosis after discharge from the hospital is very dependent on the presence of certain clinical and demographic factors. Patients at high risk of dying tend to have left ventricular dysfunction, ventricular premature beats, or an anterior infarct; mortality in these patients in the first year can be as high as 20%. Those who have none of these factors are in a relatively low-risk group, and only about 3% die in the first year. Obviously, treatment groups need to be well balanced with respect to these and other prognostic factors. Second, the mortality curves after discharge from hospital for patients who have had acute myocardial infarction clearly show increased mortality in the subsequent 6 month period; after that time, the mortality rate is approximately equivalent to that found in patients with stable coronary artery disease. Fatalities in the first 6 months after myocardial infarction are nearly all due to sudden cardiac death or recurrent infarction. It is clear that a secondary prevention trial involving patients who have had a myocardial infarction more than 6 months previously selects individuals who have already survived the greatest period of risk. On the other hand, a study that involves patients in the first few weeks after infarction selects a population initially at much higher risk, and has the potential or much greater impact in terms of the number of lives saved.

In the secondary prevention of myocardial infarction, three platelet-modifying agents have been evaluated in eight randomized double-blind clinical trials (table 1): five studies have assessed aspirin alone, one has assessed aspirin alone and aspirin in combination with dipyridamole, and two have evaluated sulfinpyrazone. Clinical trials designed to assess the effects of these agents on secondary prevention of myocardial infarction have used mortality, nonfatal recurrent myocardial infarction, and coronary incidence (coronary mortality or nonfatal myocardial infarction) as outcome events.

In 1974 Elwood et al.[2, 37] reported a randomized, double-blind study of aspirin (300 mg daily) in 1239 men with recent myocardial infarction. There was an observed risk reduction of 25% in total mortality after 1 year, but this was not statistically significant. However, for men whose qualifying infarction occurred less than 6 weeks before entry into the study, there was a statistically significant reduction in mortality from 13.2% in the placebo group to 7.8% in the aspirin group. The corresponding mortality rates in men with less recent infarction were 8.3% and 8.8%, respectively.

The Coronary Drug Project Research Group (1976) reported on 1529 men who had had a myocardial infarction some 7 years previously; these were patients who had participated in two other secondary prevention studies that had terminated prematurely.[2, 38] They were randomly assigned to receive aspirin (972 mg daily) or placebo. The observed 30% risk reduction in total mortality, from 8.5% in the placebo group to 5.8% in the aspirin group, is clinically impressive but was not statistically significant.

In 1977 Breddin et al.[2, 39] randomly allocated 946 patients (80% men), within 6 weeks of their myocardial infarction, to aspirin (1.5 g daily), a placebo (making the assessment of the benefit of aspirin double-blind), or to phenprocoumon, and followed them for up to 2 years. There was little difference in total mortality but the coronary death rate (sudden death or fatal

TABLE 1
Randomized trials of recurrent myocardial infarction

Study	Drug	Duration	No. of patients randomized	Outcome
Elwood et al., 1974[37]	300 mg/day aspirin	2½ yr	1239	Mortality reduced 25% (NS)
CDP, 1976[36]	1 g/day aspirin	2 yr	1529	Mortality reduced 30% (NS)
Breddin et al., 1977[39]	1.5 g/day aspirin	2 yr	946	NS
Elwood and Sweetnam, 1979[40]	900 mg/day aspirin	1 yr	1682	Mortality reduced 17% (NS)
AMIS, 1980[41]	1 g/day aspirin	3 yr	4524	NS
ART, 1980[45]	800 mg/day sulfinpyrazone	16 mo	1558	Sudden death reduced 57% ($p = .018$); overall mortality NS ($p = .118$)
ARIS, 1982[53]	800 mg/day sulfinpyrazone	19 mo	727	Reinfarction reduced 56% ($p < .005$); overall mortality NS
PARIS, 1980[42]	1 g/day aspirin plus 225 mg/day dipyridamole	3 yr	2206	Coronary incidence reduced 50%; overall mortality NS

CDP = Coronary Drug Project; ARIS = Anthuran Reinfarction Italian Study.

myocardial infarction), while not significantly different, was only 4.1% in the aspirin-treated patients compared with 7.1% in the placebo group.

In the randomized trial by Elwood and Sweetnam in 1979,[2, 40] most of the 1682 patients (85% men) were admitted within 1 week of their qualifying infarctions. Total mortality was reduced relatively by 17% in the aspirin (900 mg daily) group (mortality of 12.3% compared with 14.8% in the placebo group). The corresponding reduction in total mortality or nonfatal myocardial infarction was 28%; for ischemic heart disease mortality it was 22%. None of these reductions in risk was statistically significant.

The Aspirin–Myocardial Infarction Study (AMIS) Research Group (1980) carried out a multicenter, double-blind, randomized trial for which they recruited 4524 patients (89% men) who had documented myocardial infarction 2 to 60 months previously, and followed them for up to 3 years.[2, 41] The total mortality was 10.8% in the aspirin (1 g daily) group and 9.7% in the placebo group. There were fewer nonfatal myocardial infarctions in the aspirin group and the coronary incidence (coronary heart disease mortality or definite nonfatal myocardial infarction) was 14.1% in the aspirin group compared with 14.8% in the placebo group. It is unfortunate that in spite of random allocation, there was a much greater preponderance of cardiovascular risk factors among patients in the aspirin-treated group and this may contribute to part of the lack of effect for aspirin in this study.

The Persantine-Aspirin Reinfarction Study (PARIS) Research Group (1980) recruited 2206 patients (87% men) with documented myocardial infarction 2 to 60 months previously who were followed for 41 months on average.[2, 42] These patients were randomly allocated to receive either 972 mg aspirin daily, 972 mg aspirin daily plus 225 mg dipyridamole daily, or placebo, with twice as many patients in each of the active treatment groups. The total mortality, coronary heart disease mortality, cardiovascular mortality, and coronary incidence (coronary death or nonfatal myocardial infarction) were quite similar and consistently less than in the placebo group in all drug-treated groups, but the differences were not statistically significant. For coronary mortality and coronary incidence the rates were about 50% lower in the aspirin plus dipyridamole group than the placebo group from 8 to 24 months of follow-up and this difference was statistically significant by the study criteria; the corresponding reduction for the aspirin group was 30% over this period of follow-up. This should be interpreted with some caution since this particular analysis is clearly post hoc.

For the subgroup of 447 patients entered within 6 months of their qualifying infarctions, it was found that the mortality was 51% less in the aspirin group and 44% less in the aspirin plus dipyridamole group when compared with that in the placebo group (comprising only 95 patients). In contrast, for patients enrolled more than 6 months after their myocardial infarctions the observed differences in mortality were very small.

Thus, six studies have assessed the efficacy of aspirin alone in the treatment of patients after acute myocardial infarction.[2, 37–42] Five studies, incorporating a range of doses from 300 mg to 1.5 g daily, reported trends in favor of aspirin therapy with respect to several important outcomes. In contrast, the AMIS, by far the largest and statistically most definitive trial, demonstrated no effect of aspirin on any outcome event, including total coronary incidence. A subsequent analysis by Peto,[43] in which the results of all the aspirin trials were combined, has suggested that there may be a benefit of aspirin. Peto showed that for cardiovascular death, the pooled estimate of the reduction in risk with aspirin is 16% ($p < .01$); for the outcome of first infarction, fatal or nonfatal, the pooled estimate of the reduction in risk with aspirin is 21% ($p < .001$).

However, that analysis is compromised by the dissimilarities in the studies, including the variable entry time (mean of 7 days to 7 years) and dose regimen (300 to 1500 mg/day). Peto has rightly pointed out that very large trials involving 5000 to 10,000 patients per group would be required to ensure that an actual reduction in risk of 10% to 20% can be observed with confidence. Lacking such large trials, he reasoned that data from a number of properly randomized trials should be pooled to provide the best guide to the true effects of any therapeutic agent because the potential compromise may be less important than the dangers of failing to recognize a medically important effect for which evidence already exists. Since not all clinical epidemiologists agree with this approach, the present evidence for a beneficial effect of aspirin therapy in the secondary prevention of myocardial infarction is not conclusive.

The Anturane Reinfarction Trial (ART) Research Group (1978) reported the initial findings of a trial in which patients were randomly allocated to sulfinpyrazone (800 mg daily) or placebo 25 to 35 days after a myocardial infarction, and claimed a statistically significant reduction in cardiac mortality from 9.5% per annum to 4.9%.[44] Patient accession was stopped at that time and although a benefit over the first few months seemed clear, the long-term efficacy and tolerability of sulfinpyrazone could not be established with the data at hand. The trial was, therefore, continued until all 1558

eligible patients (87% men) had completed at least 1 year of follow-up. By that time the patients had been followed for 16 months on average and a second report appeared[45] in which an overall reduction in cardiac mortality of 32% was reported ($p = .06$). This reduction was due almost entirely to a 75% reduction in sudden death over the first 6 months of treatment ($p = .003$), after which time there seemed to be no further benefit of treatment.

A number of concerns have been raised about this study,[46-51] relating primarily to the general strategy adopted for the exclusion of certain patients and events from the principal analysis of efficacy, to the apparent switch of the study focus from cardiac death as the primary outcome event to sudden death, and to the multiple examinations of the data. In their response, members of the study Policy Committee have pointed out[52] that the rules relating to the eligibility of patients and the disqualification of certain outcome events were established a priori, and applied consistently to individual patients, without knowledge of which treatment group they were in. Similarly, from the start of the study, deaths had been classified, without knowledge of treatment and according to specific criteria, as myocardial infarction, or sudden or other cardiac deaths, and it had always been the intention to analyze sudden deaths separately as one of several clinically cogent subgroups of outcome. Even allowing for the multiple examinations of the data, the observed reduction in sudden death at 6 months is statistically significant by conventional standards.

In the critique of this study published by the U.S. Food and Drug Administration (FDA),[48] the principal criticisms included that (1) the exclusion of certain patients as "ineligible" and certain deaths as "nonanalyzable" was a potential source of bias, and (2) the criteria by which causes of deaths were classified were ambiguous and their application inconsistent.

After the FDA critique, the Policy Committee of ART initiated a formal review of the records by an independent committee. This committee reanalyzed all 163 deaths in both treatment and control groups in a blinded fashion.[52] The results of their review were, in general, similar to those originally reported. When all patients and all deaths were considered, the percent reduction in total cardiac death between placebo- and sulfinpyrazone-treated patients was not significantly different ($p = .118$). However, even with the reclassification of deaths and application of the intent-to-treat approach, a statistically significant 57% reduction in risk in the number of sudden deaths in the first 6 months was still observed ($p = .018$).

In a subsequent trial, the Anturan Reinfarction Italian Study Group recruited 727 patients within 15 to 25 days after acute myocardial infarction and randomly allocated them to sulfinpyrazone (800 mg daily) or placebo.[53] When analyzed on an intention-to-treat basis, there was no significant difference in total mortality in the sulfinpyrazone-treated and placebo groups. In contrast to the ART, there was no observed beneficial effect of sulfinpyrazone on incidence of sudden death. However, the number of reinfarctions, fatal or nonfatal, was significantly reduced in sulfinpyrazone-treated patients ($p < .005$). There were 34 reinfarctions in the placebo group and 15 in the sulfinpyrazone group, an observed reduction of 56%. In addition to reinfarctions, fatal and nonfatal conditions presumed to be thromboembolic event were also reduced by 66% in the sulfinpyrazone-treated patients ($p < .001$). Although the reported benefit in preventing reinfarction was consistent with the underlying antithrombotic rationale of this study design, it did not confirm the outcome of the larger ART study. The conflicting results between the studies leave many issues unresolved regarding the efficacy of sulfinpyrazone in the secondary prevention of myocardial infarction.

In PARIS, the combination of aspirin and dipyridamole showed a trend toward greater efficacy than aspirin alone in the reduction of coronary incidence during the first 2 years of therapy.[42] At the conclusion of the study (36 months) a trend in favor of either aspirin or the combination for the reduction of overall total and coronary artery–related mortality was also reported, but no difference was shown between the combination and aspirin alone. The entry of patients late after myocardial infarction may have compromised the study, since subsequent analysis indicated that a subgroup of patients receiving the combination who were entered less than 6 months after myocardial infarction had a significant reduction in 3 year coronary mortality throughout the 3 years of the study. Because the effects on overall mortality were not conclusive, a new prospective study enrolling patients between 3 weeks and 6 months after myocardial infarction was initiated (PARIS II), but the results are not presently available for evaluation. Thus, at this time there is no conclusive evidence that dipyridamole in combination with aspirin should be used for secondary prevention of myocardial infarction.

While overall these results may prompt individual physicians to recommend platelet-modifying agents to prevent reinfarction they do not warrant a general recommendation for the use of any single or combination platelet-modifying regimen.

The lack of effect may have several explanations. These include the possibilities that platelets are unimportant in the pathogenesis of coronary artery occlusion, that the platelet-modifying agent is ineffective in altering the process becauses it blocks only one of several independent pathways of platelet activation, that the doses used in the studies were inappropriate, and that the study design may have been inefficient because the number of patients was inadequate or many patients were entered late after their qualifying infarction.

In view of the current enthusiasm for β-blockers and anticoagulants and the investment of time, money, and resources required to conduct multicenter trials, it is unlikely that the unresolved issues will be settled by any new trials testing the effects of platelet-modifying agents in the secondary prevention of acute myocardial infarction in the forseeable future. A more compelling question regarding patient management may be the issue of whether β-blockers, platelet inhibitors, and the anticoagulants benefit the same or different groups of patients.

For example, aspirin inhibits platelet aggregation in vitro and ex vivo by selective irreversible acetylation of platelet cyclooxygenase,[54] thereby blocking the synthesis of prostaglandins, including the proaggregatory substance thromboxane A_2. A similar action on vessel wall cyclooxygenase concurrently decreases production of prostacyclin, the potent vasodilator and inhibitor of platelet aggregation. Since cyclooxygenase is renewable in vascular tisues but not in platelets,[55] low and infrequent doses of aspirin (1 to 2 mg/kg/day) could, in theory, inhibit platelet aggregation while sparing the capacity of the vessels to produce prostacyclin.[56] Although the clinical trials assessing aspirin in patients with acute myocardial infarction used doses sufficiently large (325 mg/day to 1.5 g/day) to inhibit both platelet and vascular wall cyclooxygenase, no dose-response effects were noted in the clinical outcome events. Moreover, long-term low-dose aspirin causes a cumulative inhibition of vascular cyclooxygenase.[56, 57] In this regard, however, recent studies of drugs that produce selective blockade of thromboxane synthetase[58] and prevent thromboxane A_2 formation without impairing prostacyclin generation have shown that these drugs fail to exhibit antithrombotic effects in experimental preparations.[59] The lack of antithrombotic efficacy of thromboxane synthetase inhibitors has been attributed to the enhanced production of the proaggregatory endoperoxides prostaglandin (PG) G_2 and PGH_2 in the absence of thromboxane A_2, but this interpretation seems false in view of recent results in primates.[60] In studies carried out in baboons aspirin has been shown to have dose-dependent antithrombotic effects independent of its inhibition of platelet cyclooxygenase when used in combination with dipyridamole or sulfinpyrazone. Thus, measurements of effects of aspirin on thromboxane A_2 production by platelets do not necessarily predict antithrombotic efficacy in vivo. Be that as it may, and despite recommendations to the contrary,[2] the aspirin dilemma will probably not be resolved soon, since the commitment in time, money, and resources required to complete such trials will be difficult to mount with the many competing therapeutic strategies being pursued in this patient population at present.

Unstable angina. There is no persuasive evidence that thrombotic mechanisms produce unstable angina. However, patients with unstable angina and recent chest pain also have higher thromboxane B_2 levels in the coronary sinus than in the ascending aorta, which suggests that recent episodes of angina in such patients are associated with local release of thromboxane A_2.[12, 16] Thus, it has been proposed that a reduction in the frequency of myocardial infarction in patients with unstable angina might be due to blockade of thromboxane A_2 production or by interference with other vasospastic processes.[10]

Lewis et al.[17] conducted a multicenter, double-blind, placebo-controlled randomized trial of 12 weeks of treatment with aspirin (324 mg in buffered solution daily) in 1266 men with unstable angina. Unstable angina was defined as that of new onset or worsening suddenly without increased activity and manifested by frequency of one or more episodes per day, duration of longer than 15 min, or occurrence at rest or during minimal activity. The frequency of death or acute myocardial infarction was reduced by 51% in the aspirin group compared with the placebo group (31 patients [5.0%] as compared with 65 [10.1%]; $p = .0005$). Similarly, the observed reduction in mortality in the aspirin group was 51% (10 patients as compared with 21 in the placebo group; $p = .054$).

This striking observed benefit of aspirin in the Veterans Administration (VA) study of patients with unstable angina has recently been corroborated by an independent Canadian study.[61] This randomized, double-blind, placebo-controlled trial compared the effects of aspirin (1300 mg daily) and sulfinpyrazone (800 mg daily), either singly or in combination, with respect to the incidence of subsequent myocardial infarction or cardiac death. Patients were followed for up to 2 years (mean 19 months) and the primary analysis of efficacy was based on eligible patients who had not

been off medication for more than 28 consecutive days preceding an outcome event. There was no observable benefit of sulfinpyrazone. However, among patients receiving aspirin there was an observed 55% reduction in risk in myocardial infarction or cardiac death compared with that in patients not receiving aspirin ($p = .004$); the corresponding reduction in risk for all deaths was 70% ($p = .004$); the corresponding reduction in risk for all deaths was 70% ($p = .005$). On an intent-to-treat basis there was a 43% reduction in risk for all deaths ($p = .036$). Observed benefits were similar for men and women.

Although inhibition of thromboxane A_2-vasospastic processes is implied, other pathogenetic mechanisms that are not confined to the vasoconstrictive action of activated platelets or nonplatelet triggers of arterial spasm might account for a beneficial effect of aspirin. For example, a reduction in ischemic episodes is reported with the use of ticlopidine,[62] an antiplatelet agent devoid of inhibitory activity on either platelet cyclooxygenase or coronary artery tone.

Aspirin (324 to 1300 mg daily) clearly reduces incidence of myocardial infarction or cardiac death in patients with unstable angina. The efficacy of aspirin in patients with unstable angina over a wide dose range without benefit of sulfinpyrazone parallels the results of aspirin in patients with TIAs and suggests the possibility of similar mechanisms. These results also confirm the notion that the effects of platelet agents vary in patients with different clinical ischemic disorders.

Saphenous vein coronary artery bypass grafts. The early clinical trials evaluating the effect of platelet-modifying drugs on graft patency were compromised by the limited size of the studies and the initiation of therapy several days after surgery when platelet deposition and thrombotic occlusion of vulnerable grafts may have already been completed.[63–67] Thus, the results were not conclusive. For example, Pantely et al.[63] examined 50 patients randomly assigned to receive a combination of aspirin and dipyridamole (325 mg aspirin and 75 mg tid dipyridamole), warfarin, or placebo. The antiplatelet therapy was begun 3 days postoperatively. No benefit of antiplatelet therapy was shown but a drug effect was clearly not excluded. However, in a preliminary report, Brown et al.[65] found a significant benefit with regard to graft patency ($p < .05$) at 1 year when placebo was compared with treatment (aspirin and aspirin plus dipyridamole) in patients with grafted vessels exceeding 1.5 mm in diameter or in vessels with flow greater than 40 ml/min. In other studies aspirin used alone and administered starting several days after surgery resulted in no significant protection.[66, 67]

Baur et al.[68] reported significant improvement in early patency rates with 200 mg qid sulfinpyrazone. Patency improved at 1 to 2 weeks after grafting from 90.9% in the placebo group to 96.2% in the treated group ($p < .05$).

In a study carried out by Lorenz et al.,[69] a significant antithrombotic effect of low-dose aspirin was reported for patients undergoing saphenous vein coronary artery bypass. In this study 60 patients were randomly assigned to receive aspirin (100 mg daily, starting 24 hr after operation) or placebo. Repeat angiography at 4 months showed reduced graft occlusion in the low-dose aspirin group (4/40 vs 17/53; $p = .012$). When the analysis was performed on the basis of intention-to-treat and all occlusive events were included, the difference was also significant ($p = .025$). This study requires independent confirmation before the treatment is considered established since the study involved a relatively small number of patients, some of whom had also been subjected to thromboendarterectomy, and since the untreated control group had an exceptionally high frequency of graft occlusion compared with the experience at most other centers around the world.

The combination of aspirin and dipyridamole has been convincingly shown by Chesebro et al.[70, 71] to prevent both early and late postoperative occlusion of aortocoronary artery bypass grafts. The design of this study was based on preliminary animal experiments showing efficacy of the drug combination in the reduction of platelet deposition on vein grafts[72] and formation of intimal lesions.[73] Moreover, the aspirin and dipyridamole combination has been shown to synergistically normalize survival of platelets in patients with coronary atherosclerosis[74] and in animal preparations of arterial thrombosis.[60] The randomized double-blind trial in 407 patients compared placebo with dipyridamole administered 2 days before operation plus aspirin (325 mg) begun 7 hr after operation and therapy was continued with dipyridamole at a lower dose (225 mg daily) in combination with the aspirin (975 mg daily) thereafter. Vein graft angiographic examinations were performed in 360 patients (88%) within 6 months of operation (median of 8 days). Within 1 month of operation, 3% of vein graft distal anastomoses (10 of 351) were occluded in the treated patients vs 10% (38 of 362) in the placebo group; the proportion of patients with one or more distal anastomoses occluded was 8% (10 of 130) in the treated group and 21% (27 of 130) in the placebo group. Early postoperative bleeding was similar in the two groups. In this trial dipyridamole and aspirin were effective ($p < .001$) in preventing graft occlusion early after oper-

ation. Although previous investigators reported less or no benefit using this combination, none had initiated antiplatelet therapy before bypass graft surgery.

In the follow-up study,[71] vein graft angiography was performed in 343 patients (84%) 6 to 18 months (median of 12 months) after operation. Eleven percent of 478 vein graft distal anastomoses were occluded in the treated group, and 25% of 486 were occluded in the placebo group. The proportion of patients with one or more distal anastomoses occluded was 22% of 171 patients in the treated group and 47% of 172 in the placebo group. All grafts were patent within a month of operation in 94 patients in the placebo group and 116 patients in the treated group; late development of occlusion was reduced from 27% in the placebo group to 16% in the treated group. The results show that the combination of dipyridamole plus aspirin continued to be effective in preventing vein graft occlusion late after operation. Thus, the combined regimen of aspirin (325 mg) and dipyridamole (75 mg) three times daily appears to be effective in patients who have undergone bypass graft surgery for the prevention of both early and late graft occlusion, and treatment should be continued for at least 1 year. However, the relative importance of the drug combination compared with either agent used singly remains to be determined.

To resolve the question of relative efficacy for the various regimens reported to show benefit, the VA Cooperative Group has organized a double-blind, randomized multicenter clinical trial. Participants are treated for 1 year. The population consists of male VA patients with angiographically documented coronary artery disease who have agreed to undergo CABG. Patients are being randomly assigned to one of the five following treatment groups: (1) 325 mg aspirin once daily, (2) 325 mg aspirin three times daily, (3) aspirin and dipyridamole (325 mg and 75 mg tid, respectively, together as a combination, (4) 267 mg tid sulfinpyrazone, and (5) placebo three times a day, continued for 1 year. Dipyridamole and sulfinpyrazone treatment is begun 2 days before surgery. In the treatment groups receiving aspirin alone or in combination, one aspirin tablet (325 mg) is administered 12 hr before surgery. Treatment is resumed 6 to 8 hr after surgery with the drugs given by nasogastric tube or orally. The primary outcome measures are the percent of occluded grafts at 1 week and at 1 year after CABG.

Membranoproliferative glomerulonephritis. Donadio et al.[23] carried out a randomized controlled clinical trial in 40 patients. Treatment for 1 year with a combination of aspirin (325 mg tid) and dipyridamole (75 mg tid) was compared with placebo. Baseline half-life of ^{51}Cr-labeled platelets was reduced in 12 of 17 patients. The platelet half-life became longer and renal function stabilized in the treated group as compared with the placebo group, suggesting a relationship between platelet consumption and the glomerulopathy. The glomerular filtration rate was better maintained in the treated group (average decrease, 1.3 ml/min/1.73 m^2 of body surface area per 12 months) than in the placebo group (average decrease 19.6). Fewer patients in the treated group than in the placebo group had progression to end-stage renal disease. The data suggest that the therapy slowed the deterioration of renal function and the development of end-stage renal disease. In the management of this disorder the relative importance of the drug combination compared with the drugs used singly or at different dosages remains to be determined.

Prosthetic heart valves. Thromboembolism continues to be a significant complication in patients with artificial heart valves, although the risks have been reduced considerably during the past several years by improved design of prostheses. In patients with mechanical heart valves, the overall incidence of clinically detectable thromboembolism for mitral Starr-Edwards prostheses at the Mayo Clinic over a 5 year period of follow-up was 25%. The important predictors of thromboembolism were the adequacy of anticoagulant therapy, the presence of left atrial thrombus at time of operation, and a large left atrium. On the other had, the aortic Starr-Edwards prosthesis carried a 5 year incidence of thromboembolism of less than 10%.[75] In patients with Björk-Shiley prostheses, the incidence of thromboembolism may be somewhat lower than in patients with Starr-Edwards prostheses.[76] In the experience of the majority of clinicians, inadequate anticoagulant control has been the principal factor predisposing to clinically significant thrombosis of most prosthetic heart valves of recent design.[75]

Six prospective randomized trials have evaluated the effects of platelet-suppressive drugs in patients with prosthetic heart valves.[77-82]

Sullivan et al.[77] assessed the benefit of dipyridamole (400 mg daily) in patients with prosthetic heart valves of the older type in the aortic and mitral position. This was a well-designed, randomized double-blind trial in which 163 patients received either placebo or dipyridamole and all received oral anticoagulants. During the 1 year of observation, emboli developed in 14% of the placebo-treated patients as compared with 1.3% of those treated with dipyridamole. Dipyridamole at doses of about 300 to 400 mg/day as a supplement to anticoagulant therapy also effectively reduced thromboembolism in three additional trials,[78-80] although the

results in one study[80] did not reach statistical significance, perhaps because of the low frequency of thromboembolism.

Aspirin also appears to be effective in reducing thromboembolic complications of prosthetic heart valves, although this drug has been studied in fewer patients.[81, 82] Dale et al.[81] observed a reduced incidence of thromboembolism in patients receiving both an oral anticoagulant and aspirin (1 g daily) compared with that in those on oral anticoagulant therapy only. Unfortunately, the incidence of gastrointestinal bleeding was high in the group on aspirin plus oral antaicoagulant. Altman et al.[82] gave aspirin (500 mg daily) with oral anticoagulants and reported a significantly reduced incidence of thromboembolism without any increase in the frequency of bleeding. In contrast, however, subsequent experience with oral antaicoagulants plus lower dose aspirin (500 mg daily) in patients who had mechanical heart valves showed excessive gastrointestinal bleeding that was judged to be prohibitive in the group receiving reduced doses of aspirin as well.[75]

Based on the currently available information, patients with mechanical heart valves should receive a combination of anticoagulants and 400 mg/day dipyridamole. Sulfinpyrazone may be a possible substitute. Aspirin, however, is not recommended because of the high frequency of gastrointestinal bleeding.

Arteriovenous silicone rubber cannulae. In a well-designed randomized trial of 600 mg sulfinpyrazone daily,[83] the number of patients developing thrombi was significantly less in the group given sulfinpyrazone than in those receiving placebo, and there was a fourfold difference in the number of thrombi per patient per month between the two groups. This study was extended with a crossover at 6 months to the opposite treatment for another 6 months. The significant difference in favor of sulfinpyrazone was reproduced. The time course of thrombotic events suggested that drug therapy was effective within a week of commencing treatment.

Harter et al.[84] reported similar results with low-dose aspirin. While this study provides evidence for the antithrombotic efficacy of low-dose aspirin, that conclusion may only be valid when there is associated platelet dysfunction[27] and may not apply to patients with normal platelet function.

Although the reduction in occlusive events for sulfinpyrazone and aspirin are statistically different, the effects have not been sufficiently striking to have much clinical impact on the management of these patients. Moreover, arteriovenous cannulae are only rarely used at present to provide vascular access for patients undergoing chronic hemodialysis therapy for renal failure. Thus, the clinical importance of these results is largely theoretical.

Prosthetic vascular grafts. Vascular grafts have been used in larger blood vessels in humans for several decades with a relatively high degree of success. At present, most successful vascular graft materials are knitted or woven or are fabricated from microporous materials. Prosthetic vascular grafts often fail because of excessive deposition of thrombus on the luminal thrombogenic surface or embolization from that thrombogenic surface. The junction of a gaft with the blood vessel wall appears particularly prone to thrombus formation, perhaps due to chronic endothelial injury and denudation together with the luminal narrowing secondary to intimal thickening. By and large, vascular grafts in blood vessels with an internal diameter of less than 5 mm become occluded despite use of improved materials and antiplatelet agents. Presumably, biomaterials with (1) little or no reactivity to flowing blood, (2) characteristics that promote endothelial coverage, and (3) inhibit anastomotic intimal stenosis will be required if grafts are to be used successfully in blood vessels with an internal diameter of less than 5 mm.

^{111}In-platelet imaging studies in patients with prosthetic vascular grafts have shown ongoing platelet deposition continuing for many years.[85] This technique has also been used to assess the capacity of antiplatelet therapy to reduce platelet deposition. The combination of aspirin and dipyridamole has been reported to reduce platelet deposition in patients with femoropopliteal grafts.[86] Suoloctidil and ticlopidine have been reported to be ineffective.[87] Since the effect of aspirin and dipyridamole on the long-term patency of prosthetic grafts in man is not completely known.[88-90] no general recommendation can be made at present. This problem is under active investigation.

Drug evaluation

Aspirin. Aspirin, a nonsteroidal anti-inflammatory agent, potently and irreversibly inactivates platelet cycloxygenase by aceteylation.[54] While all of aspirin's antithrombotic effects have been attributed to this blockade of thromboxane A_2 formation by platelets, there is evidence for antithrombotic effects independent of its inactivation of cyclooxygenase.[60]

At present there is intense interest in aspirin therapy for vascular disease.[1-3, 56] Aspirin has been shown in well-designed clinical trials to be beneficial in at least five and possibly six clinical settings relevant to cardiovascular disease (table 2), as follows: (1) reduction of thromboembolic complications associated with arti-

TABLE 2
Positive clinical trials of aspirin

Prosthetic heart valves (with anticoagulants)
Dale et al. (1977)[81]
TIAs
Aspirin in TIAs (1977)[18]
Canadian Cooperative Study Group (1978)[19]
Arteriovenous cannula (with platelet dysfunction)
Harter et al. (1979)[84]
Unstable angina
Lewis et al. (1983)[17, 61]
Saphenous vein coronary artery bypass
Lorenz et al. (1984)[69]

ficial heart valves,[81, 82] (2) prevention of stroke and death in patients with TIAs,[18, 19] (3) decrease in thrombotic occlusion of arteriovenous silicone rubber cannulae in uremic patients undergoing hemodialysis,[84] (4) reductions in incidence of myocardial infarction and cardiac death in patients with unstable angina,[17, 61] and (5) possibly a decrease in venous thrombosis in men after hip replacement.[91] Although aspirin has recently also been reported to increase the patency of saphenous vein coronary bypass grafts,[69] this report requires confirmation.

These reported benefits of aspirin require some comment. First, the antithrombotic effects of aspirin in association with oral anticoagulant therapy in patients with prosthetic mitral valves[81, 82] are evident, but this combination is associated with an unacceptably high frequency of gastrointestinal bleeding.[75] Second, the capacity of aspirin in low doses to reduce the thrombotic complications associated with arteriovenous cannulae[84] has been shown in patients undergoing long-term hemodialysis, and thus with significant platelet dysfunction. It cannot be assumed that a similar dose would be antithrombotic in the absence of associated platelet dysfunction. Third, the pathogenetic mechanisms of TIAs and unstable angina may not be thrombotic but vasospastic. Thus, the benefits of aspirin in those clinical settings[17–19, 61] may reflect blockade of thromboxane A_2–mediated vasospasm rather than inhibition of platelet-dependent thrombus formation.

Sulfinpyrazone. Sulfinpyrazone is a urocosuric agent with weak anti-inflammatory properties that is without a defined mechanism of antithrombotic action,[2, 60, 92–94] although a protective effect on reducing endothelial injury has been postulated.[95] There may be positive antithrombotic effects of the combination of aspirin and sulfinpyrazone.[18, 60]

Sulfinpyrazone reduces incidence of occlusion of arteriovenous cannulae[83] and early occlusion of coronary artery saphenous vein grafts.[68] Since the claimed benefit with respect to reducing mortality and the secondary prevention of myocardial infarction[44–53] remains controversial, no recommendations can be made regarding that possible indication. The report that it may also decrease thromboembolism in patients with substitute heart valves[75] requires confirmation.

Dipyridamole. Dipyridamole, a coronary vasodilator with weak inhibitory effects on phosphodiesterase activity,[2, 60] appears to increase inhibitory cyclic AMP levels in platelets by elevating blood adenosine levels through the blockade of adenosine uptake by red cells and vascular wall cells.[96–99] Aspirin potentiates the antithrombotic effects of dipyridamole in the baboon by mechanisms independent of inactivation of platelet cyclooxygenase.[60]

Dipyridamole (in combination with anticoagulants) decreases thromboembolism in patients with artificial heart valves[77–80] and in combination with aspirin it has been shown to reduce both early and late coronary artery saphenous vein graft occlusion[70, 71] and preserve renal function in patients with membranoproliferative glomerulonephritis.[23] The importance of dipyridamole in combination with aspirin remains to be established.

Ticlopidine. Of the antiplatelet drugs currently available for clinical investigation, ticlopidine is one of the most potent and has several important advantages over existing drugs.[35, 100] Ticlopidine is chemically unrelated to other antiplatelet drugs and appears to have a unique, albeit unknown, mechanism of action. It is neither a prostaglandin synthesis inhibitor, nor a cyclic AMP phosphodiesterase inhibitor. There are several reports that indicate that ticlopidine may act on the platelet membrane to alter its reactivity to activating stimuli.[100] The broad spectrum of antiplatelet activity of ticlopidine and its apparently novel mechanism of action sets the drug apart from currently available antiplatelet agents. The fact that ticlopidine does not inhibit prostacyclin synthesis in the arterial wall, but can still inhibit aggregation induced by thromboxane A_2 and prostaglandin endoperoxide, is a theoretical advantage over aspirin.

A number of important well-designed multicenter trials are ongoing. These trials should determine the ultimate clinical role of ticlopidine.

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Research in Russia on Vitamins and Atherosclerosis

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THE EFFECTS of vitamins on atherogenesis and its clinical complications have been investigated extensively in Russia during the past 15 years. The importance of certain vitamins in the treatment of coronary heart disease and other manifestations of severe atherosclerosis seems to be generally accepted. Since some of these views are not held in the United States and other Western countries, it is of interest to present here a review of the pertinent Russian literature. The references cited are representative rather than complete, though we have attempted to include the most important studies. No attempt is made here to cover the corresponding Western literature.

Research Approaches and Methods in Russia

Several approaches have been used by Russian investigators and a general comment on their validity and limitations may be useful before reviewing specific results. The Russian work is concerned with pharmacologic dosages of vitamins, usually far in excess of normal average vitamin intakes and requirements for nutritional purposes, and not with vitamin deficiencies or variations in ordinary diets. This explains, perhaps, the absence of epidemiologic studies, such as have been done elsewhere in connection with the diet.[1-4]

The evidence presented for the effects of the vitamins comes from two general sources—experiments on laboratory animals, largely confined to rabbits, and observations on the clinical course of patients with coronary heart disease treated with vitamins.

The clinical studies on this subject in Russia are subject to serious limitations. Spontaneous variability in clinical status and in the severity and frequency of symptoms is notorious in patients with coronary heart disease. The use of these criteria, particularly subjective reports of angina pectoris, is difficult and hazardous under the best of circumstances. The double-blind experiment, with a placebo control, which we now consider to be essential in the evaluation of drug therapy where subjective criteria are involved, does not seem to have been used in any of the Russian studies.

It is not even clear that other treatment, including dietary management, was discontinued or kept absolutely constant during the trials with vitamins but it does appear that the patients knew when they were being given vitamin therapy. Since a large proportion of patients with coronary heart disease report benefit from any treatment, including placebos, it is not surprising than many clinical reports of favorable action of the vitamins are found in the literature.

Finally, in the clinical studies on vitamins, as in most medical research in Russia, inadequate attention is given to the requirements for statistical analysis, as was noted previously.[5,6] This is true of the reports concerned with objective criteria as well as those in which only subjectiv criteria were involved. Though the data required for statistical analysis are generally absent, it is obvious that almost all of the clinical samples were far too small to yield significance of the

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reported subjective improvement unless really spectacular benefit resulted from the vitamin therapy.

Electrocardiographic evidence of effects of vitamin therapy is claimed in some papers but there is no basis whatever to judge acceptability or significance of the evidence. Qualitative observations on some of the patients but no measurements of electrocardiographic items are reported. In a minority of the reports crude frequency distributions of qualitative electrocardiographic characteristics are given with no attempt at statistical evaluation.

The most acceptable evidence of vitamin effects in the Russian investigations on patients is provided by measurements of cholesterol and other lipids in the blood. While a decrease in cholesterol concentration in the blood does not necessarily mean clinical benefit, in general it will be agreed that this would be a desirable change and this criterion has been much used in Russia. Most Russian workers have used the Engelhardt-Smirnova method* for the estimation of cholesterol in studies both on animals and on patients. We have been unable to obtain detailed data on the reliability and validity of this method but it seems to give values in the range, though perhaps somewhat lower, that we would expect. These questions are not serious, however, when the same method is applied to the same patients in therapeutic trials.

Many of the Russian papers report data on plasma or serum "lecithin." It appears that this item is measured as lipid-soluble phosphorus and that "lecithin" is simply phospholipid. This measurement is widely considered to be significant, and emphasis is given frequently to the cholesterol/lecithin ratio though no statistically valid evidence is presented that the phospholipid value adds significantly to the information given by the cholesterol measurement.

Anitchkov's studies on the experimental production of atherosclerosis in rabbits span half a century[7-12] and the sense of priority in this field is strong in Russia. During all this time Anitchkov has insisted, and his opinion is generally accepted in Russia, that these experimental lesions are true replicas of atherosclerosis in man. The statement is made[10] that the atherosclerosis produced in rabbits is not only similar but substantially identical with that seen in man, especially when small levels of cholesterol are fed so that the development of the lesion is slow.[7] It should be noted, however, that most of the Russian studies have used large dosages and short periods (3 to 4 months). Recently, atherosclerosis has been produced in dogs by cholesterol feeding plus thiouracil in Russia, and this too is considered to be comparable to the human lesion.[13, 14]

The rabbit is generally preferred to chickens and dogs as the experimental animal.[15] Almost all animal experiments with vitamins have involved rabbits fed large doses of cholesterol so as to produce a tremendous degree of hypercholesteremia and marked lesions in the arteries within 3 to 4 months. The experiments on animals have been rigidly controlled as a rule.

Besides measurement of cholesterol and "lecithin," a major criterion of vitamin effects on atherosclerosis in the experimental animal has been visual inspection of the aorta, sometimes after staining with Sudan red. Anitchkov[11] considered this to be only a crude criterion, useful to detect gross differences, and preferred measurement of the lipid extracted from the aorta. In the absence of convenient and accurate methods, however, the Russian studies with vitamins have not utilized such measurements.

It is well known from autopsy studies on man that there is only a low degree of correlation between the severity of atherosclerosis in the aorta and that in the coronary arteries. Nevertheless, in the experimental studies in Russia with the vitamins the pathologic examinations have largely been confined to the aorta. Recently, Miasnikov[13, 17] reported atherosclerosis in the coronary arteries as well as in the aortas of 22 rabbits that had been fed cholesterol for several months. Infarction the

*Described in Prodtschark , V. M. Borovskaia and L. T. Margulina. Bakterechnipo Intersternina Metodam Issledovania. Moskva, 1950.

occur with the cholesterol feeding alone the addition of thrombin injections, pituin, or extreme exercise produced areas of rosis in the myocardium of some rabbits. e influence of simultaneous vitamin treatnt in these situations has not been reported. Fedoseev[13, 14] reported spontaneous electrodiographic changes in dogs treated with iouracil and fed massive doses of cholesrol. Atherosclerosis both in the aorta and in coronary arteries, but not infarction, was served in dogs treated in this way.[15]

As mentioned above, statistical tests of significance were not made in the Russian studies atherosclerosis. We have supplied these for their studies where sufficient data were ported.

Vitamin C

The relationship of this vitamin, as well as itamin A, to the problem of atherosclerosis obably was studied first by I. A. Miasnikov,[13] who used rabbits fed massive doses of holesterol. Her original publication is not ssible to us but the essential results were produced by A. L. Miasnikov in 1954.[15] The nclusion was that vitamin A accentuates nd vitamin C inhibits the atherosclerosis proced by cholesterol feeding. In another early aper (1952) it was reported that treatment ith ascorbic acid delays the serum choles- rol rise in rabbits fed cholesterol.[22] ese findings were quickly applied to clinical trials. In 1952 Tiapina[21] intravenously administered 500 mg. of ascorbic acid daily for several weeks to patients with coronary heart disease and reported a substantial decrease in the serum cholesterol concentration, as much as 150 mg. per 100 ml. in one patient. Later (1954) A. L. Miasnikov[23] reported that ascorbic acid lowered the hypercholesteremia of cholesterol-fed rabbits. The data are inadequate to judge whether the difference was statistically significant. Loviagina's and Sinitsyn's data[26] show high degree of variability in the serum cholesterol levels of rabbits fed with cholesterol for 105 days.

The treatment of patients with coronary heart disease by intravenous solution of ascorbic acid gained popularity quickly and in 1956 Sedov[24, 25] reported the results with 106 patients. They received 500 to 1,000 mg. daily for 20 to 30 days, and the treatment was repeated after an interval of 1 to 3 months. Before the treatment 23 per cent of the patients were stated to have had serum cholesterol values over 250 mg. per 100 ml., but after the treatment only 2.3 per cent had values that high. We find the reported cholesterol change is highly significant ($p < 0.0001$ by chi-square). The cholesterol level was unaffected in the patients who initially had low values, a finding in harmony with I. A. Miasnikova's work in 1947.[18] Sedov reported clinical improvement but gave no specific data.

According to Sedov,[25] the serum cholesterol level falls during the first days of treatment with ascorbic acid and is kept at a depressed level with chronic medication.

A very rapid response to intravenous ascorbic acid, with a fall in the serum cholesterol within a few hours, was reported by Tiapina,[26] but this was not confirmed by Grabenko.[27] Both Grabenko and Tiapina agreed with the other Russian workers, however, that treatment continued for several weeks or months lowers the blood cholesterol. The results obtained by Fedorova (quoted by Miasnikov[23]) were less uniform, with only 60 per cent of the patients showing a fall, 30 per cent no change, and 10 per cent an increase. According to Miasnikov,[23] a decrease in the blood cholesterol is obtained only with prolonged administration of ascorbic acid, and even then complete correction of hypercholesteremia may not be possible. Miasnikov[23] reported Tiapina's findings of an increase in the fecal cholesterol excretion in parallel with the decrease in the blood, but the details of chemical analysis were not given and no judgment is possible of what was actually measured in the feces.

Lobova[28] found that daily intravenous administration of 1,000 mg. of ascorbic acid for 10 days produced a decrease in the beta and an increase in the alpha lipoproteins in 18 among 35 patients. The responders were stated to be in an early phase of the disease, while

the 17 refractory patients were described as being in a more advanced phase.

There are some discrepancies in the reports of clinical benefits. Various studies report subjective improvement in feeling and work capacity with no specific data on controls. Lobova[29] reported that ascorbic acid improved the work capacity without change in the severity or frequency of angina. Fedorova (reported by Miasnikov[28]) found fewer anginal attacks in patients on ascorbic acid therapy.

Ascorbic acid is recommended as an auxiliary in the treatment of patients with coronary artery disease by such leading Russian cardiologists as Miasnikov,[28, 29, 30, 31] Il'inskii,[32] and Ryss.[33] The daily dosage advised is 500 to 1,000 mg. orally or 300 to 500 mg. intravenously (in glucose or physiologic saline solution). With the intravenous therapy the recommendation is a series of 20 to 30 daily injections to be repeated at intervals of 2 to 3 months. A favorable feature is stated to be that in the doses used ascorbic acid does not affect blood coagulation.[28, 34, 35]

Miasnikov,[28] writing in 1960, with the authority of a Member of the Academy of Medical Sciences and Director of the Institute of Experimental Therapeutics in Moscow, states, "Presently, ascorbic acid is widely used for prevention and therapy of atherosclerosis." He suggests also, without providing any data, that the [illegible] of people in the mountains of the Caucasus may be related to the high ascorbic acid content of their diet. Further, he states that the inhibiting effect of ascorbic acid on atherogenesis can be increased by combination with vitamin P, but the dosage and data are not provided.

If ascorbic acid inhibits hypercholesteremia and atherosclerosis, it might be expected that these conditions would be promoted by a deficiency of vitamin C in the diet. This was reported by Willis[36] to be the case in guinea pigs, and Tolmachev,[37] whose paper is not available to us, stated that scorbutic patients have an elevated level of cholesterol in the blood. Studies on this aspect have been little cultivated in Russia and the information is scanty. In the Western literature atherosclerosis has not been reported to be a prominent feature in persons suffering from vitamin C deficiency.[38-40]

When one of us (A. K.) returned from Russia in 1956 with the information about the enthusiasm there about ascorbic acid, full controlled experiments were initiated with patients at a Minnesota State Hospital on rigidly fixed diets with and without the addition of 1,000 mg. of ascorbic acid daily in alternating periods of 3 to 4 weeks at a time.[41] Though the first trial suggested the possibility of a slight effect on the serum cholesterol concentration, more careful analysis and repetitions with various base diets failed to show any indication of a statistically significant effect, at average cholesterol levels between 160 and 200 mg. per cent, dependent on the fats in the diet. This does not necessarily contradict Sedov's results,[25] who found a decrease of cholesterol only at initial levels over 200 mg. per cent. This treatment has not, as far as we know, been applied in the Western World to patients with coronary heart disease.

Vitamin A

In I. A. Miasnikova's early experiments with vitamin A it was reported that atherogenesis in rabbits fed cholesterol is promoted by the administration of vitamin A. In the early fifties restriction of vitamin A in the diet was recommended on this account,[28] but later studies did not give consistent results, and in his latest (1960) review of the subject Miasnikov[28] states that vitamin A may be disregarded in regard to prevention or treatment of patients with atherosclerosis. Ryss[33] holds that there is a complex relationship between vitamin A and cholesterol metabolism. It is suggested that hypercholesteremia may be produced by vitamin A administration as a result of eliminating cholesterol from the brain and liver.

Vitamin D_2

It appears that the effect of vitamin D_2, or calciferol, on atherogenesis is generally agreed in Russia to be unfavorable. Rabbits receiving 200 mg. of cholesterol in the diet for 90 days showed increases of 300 to

per cent in the serum choles[illegible]level and de 2 to grade 3 atherosclerosis in the aor-. The addition of 100,000 units of vitamin (0.25 mg. of crystalline calciferol) to the ly diet produced an average increase of per cent in the serum cholesterol and st of the rabbits exhibited grade 4 atherosclerosis in the aorta (Bavina[42]). We have n unable to find more recent reports in the ssian literature, but restriction of vitamin is generally recommended to patients with onary heart disease.[28, 32]

amins

According to Miasnikov[28] a large series of dies in the Institute of Experimental erapeutics in Moscow on cholesterol-fed bbits and on patients with coronary heart ease revealed no effects of thiamine administration. In peripheral arteriosclerosis, however, Gordon[43] has reported some improvement after thiamine treatment for several nths as indicated by an increase in the skin mperature.

Ryss,[33] after a thorough review of the literature, recommends administration of thiamine as a part of the "complex therapy of ronic heart failure," mainly because of the ults of studies by Kryzhanovskaia.[44]

otinic Acid

Iakovleva[45] reported that the administration of nicotinic acid to cholesterol-fed rabbits increases the hypercholesteremia and decreases atherosclerosis. Since the reports of Kuchul et al.[46] and Parsons and Finn,[47] in 55 and 1957, it has been well known in the estern world that massive doses of nicotinic id tend to depress the serum cholesterol vel in man and in some but not all species of imals. At present nicotinic acid, in oral es of 1,000 to 2,000 mg. daily, is widely ed in the United States to reduce the serum ol sterol level in patients and in persons thout complaints but who have marked hyercholesteremia.

In Russia, clinical benefit was reported by ov[48] and by Ratner et al.[49] for patients th coronary heart disease who received from 1 to 6 mg. of nicotinic acid four to six times ily. Ratner et al. stated that this treatment may prevent a final electrocardiographic responses to exercise.

Soibel[50] injected subcutaneously 5 to 10 ml. of an 0.86 per cent solution of nicotinic acid (43 to 172 mg. daily) in addition to an oral dose of 100 mg. This regimen was maintained for several weeks with 125 patients suffering from coronary insufficiency, 75 of whom reported significant subjective improvement, 36 moderate improvement, and only 10 reported little benefit. From serial electrocardiograms it was judged that 62 patients were considerably improved, 39 had a moderate improvement, the improvement was little in 17, and only six patients showed no change. The "improvements" were mainly in reduction in S-T depression; T-wave inversion and arrhythmias were less affected. Serum cholesterol was not reported but it was stated that the prothrombin time was increased in some patients.

In the Russian literature up to the time of writing this review (March 1961) massive dosage of nicotinic acid as used in the United States has not been studied, and it is obvious that treatment with nicotinic acid is considered to be in the experimental stage. It is not generally recommended as a part of the treatment of patients with the clinical complications of atherosclerosis.

Pyridoxine (Vitamin B_6)

The development of arterial lesions, quite different from spontaneous human atherosclerosis, was reported a dozen years ago in the United States in monkeys and dogs fed diets grossly deficient in pyridoxine (Rhinehart and Greenberg).[51, 52] Suggestions that this may have some bearing on human atherogenesis have been discounted by almost all workers in the West. No marked effects were found on blood cholesterol in pyridoxine deficiency in dogs or various species of monkeys.[53] Parenteral administration of 25 mg. of pyridoxine daily to rabbits did not alter the serum cholesterol level. Recently, El Sikh-Ali[54] reported moderate decreases of serum cholesterol in the majority of 13 patients with coronary heart disease who received intravenous injections of 50 to 200 mg. of pyridoxine.

There has been little interest in Russia on

this subject, judging from the literature, and it is not considered in the treatment of patients with coronary heart disease.

Vitamin B_{12}

Considerable interest in the effect of vitamin B_{12} on atherosclerosis has developed recently in Russia. Lukomskii[55] gave 20 gamma of vitamin B_{12} daily for 10 days to each of 18 patients with coronary heart disease and reported an average decrease of 23 mg. per 100 ml. in the serum cholesterol concentration, associated with a small decrease in the beta lipoproteins in the serum. The effect was stated to be enhanced when choline was given simultaneously. Motovilova[56] injected larger doses (50 gamma daily) for 10 days in 40 patients, and obtained an average fall of 18 mg. of cholesterol per 100 ml. of serum. We have analyzed the data statistically and find this small change is significant ($p = 0.003$).

The effect in Motovilova's patients was stated to be maintained for 2 months and to be accompanied by reduced frequency and severity of angina "in the majority" of the patients. Further it is stated that the electrocardiogram improved in some patients and became "normal" in five of them. Specific detailed data were not given on the clinical findings.

Fomina[57] provided a treatment consisting of injection of 30 to 50 gamma of vitamin B_{12} alternating every other day with 15 to 25 mg. of testosterone. It is stated that among 43 patients with coronary heart disease, angina pectoris disappeared in 26, and 29 patients showed a decrease in the serum cholesterol level, which averaged 62 mg. per 100 ml. in these patients. Five patients had increased serum levels (average + 36 mg. per 100 ml.), and there was no change in three patients. Our calculations indicate that the distribution of these cholesterol changes, i.e., the greater frequency of a decrease, is statistically significant. No studies were made on testosterone and vitamin B_{12} separately.

The effect of vitamin B_{12} in rabbits fed 200 mg. of cholesterol per Kg. daily was studied by Ignatova,[58, 59] who reported reduced hypercholesteremia and delayed atherogenesis. When the cholesterol feeding was discontinued after 105 days, the regressive changes were followed with two groups of nine rabbits each roughly matched in regard to serum cholesterol level.[60] The serum cholesterol concentration in the control group fell from 787 to 28 mg. per 100 ml. in 112 days, while the value in the group receiving vitamin B_{12} fell from 740 to 142 mg. per 100 ml. Individual data and statistical analysis are not reported but it seems probable that there was a significant difference between the two groups in the regression of hypercholesteremia.

Ignatova[59] made planimetric measurements of the areas of the lesions in the aortas stained with Sudan III and examined sections of the walls microscopically from which it was concluded that the severity of the atherosclerosis was considerably greater in the control rabbits than in the rabbits treated with vitamin B_{12}.*

On the basis of these results, Miasnikov[25] recommends the use of vitamin B_{12} in a dosage of 0.6 mg. every second day for 2 to 4 weeks for prevention and treatment of atherosclerosis, though he indicates that clinical experience is as yet too limited for conclusive evaluation. Motovilova[56] found the prothrombin content of the blood increased in 11 out of 23 patients treated with vitamin B_{12}. Statistical analysis of her results shows an average increase of 10 per cent in the blood prothrombin, and this was statistically highly significant. It may appear, therefore, that treatment with vitamin B_{12} would be questionable unless combined with anticoagulant treatment.

Choline

In the Russian literature on treatment of patients with atherosclerosis choline is considered together with vitamin therapy.[25, 55, 56] In the Western World enthusiastic reports,

* In her latest study, Ignatova[59] found that daily intramuscular injection of 100 gamma vitamin B_{12} for 8 weeks in 75 patients with coronary atherosclerosis decreased the blood cholesterol by about 20 per cent on the average. We found the distribution of decreased cholesterol in 69 of 75 patients statistically significant. Changes of blood coagulation occurred in some patients.

this therapy. According to [illegible]ikov[illegible] it is widespread. It appears likely, too, that treatment with vitamin B_{12} will receive acceptance. It is curious, however, that massive dosage with nicotinic acid, currently so popular in the United States, has as yet been given little attention in Russia.

The possible mechanisms involved in the reported effects of the vitamins are discussed at length by Russian authors but a presentation of these considerations, some quite speculative, would require far more space than is appropriate to a review devoted to the basic clinical and experimental reports. Obviously, the reports reviewed here require careful consideration. Repetition of some of the studies, with more attention to controls and statistical analysis would be essential for critical evaluation. The great contributions of the pioneering work in Russia on atherosclerosis are obvious to all, and we may be sure that valuable research will continue in that country.

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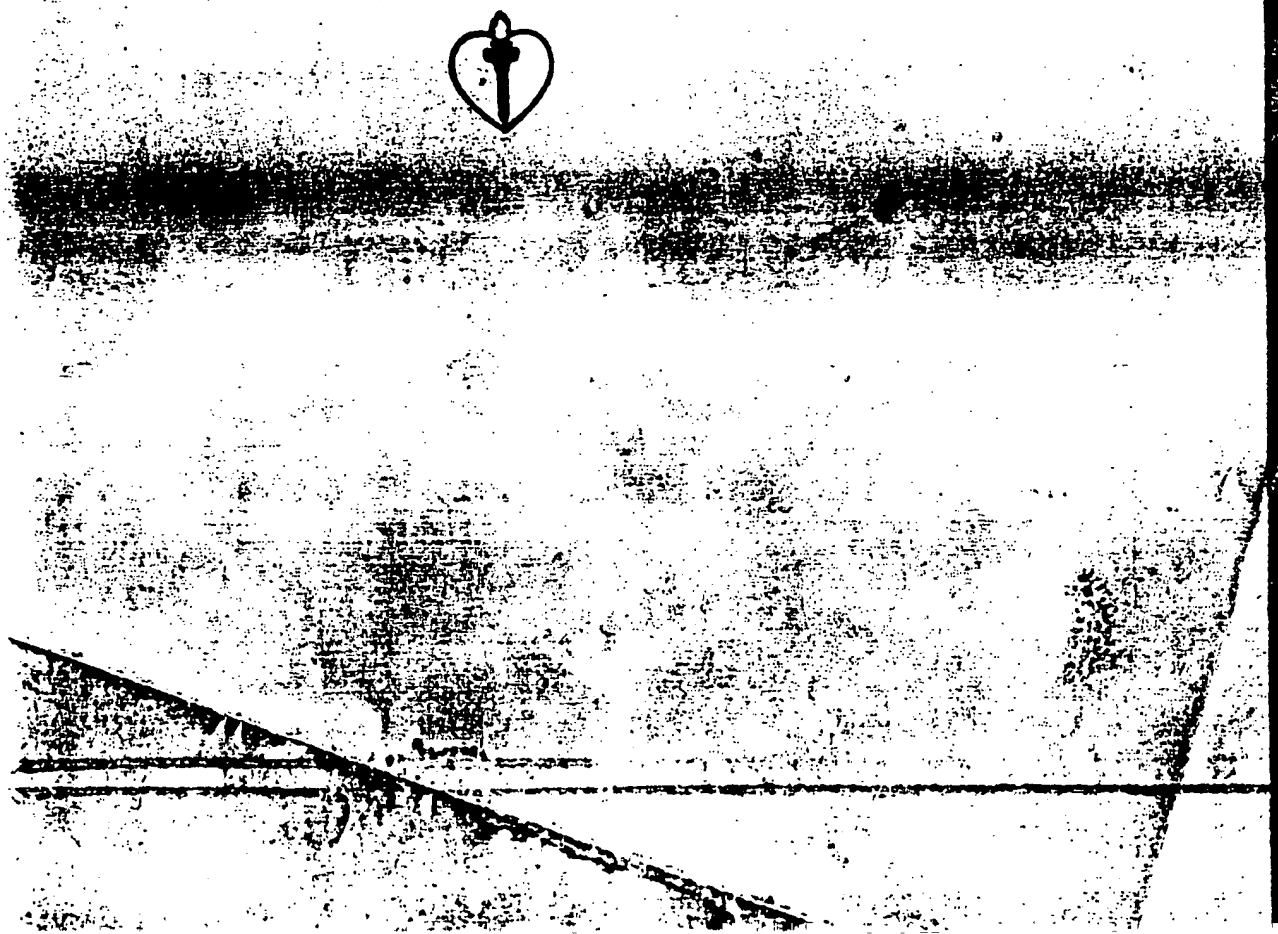

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The RIGHT DOSE

How to Take Vitamins & Minerals Safely

By Patricia Hausman, M.S.
Author of THE CALCIUM BIBLE

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Notice

This book is intended as a reference volume only, not as a medical manual or a guide to self-treatment. If you suspect that you have a medical problem, we urge you to seek competent medical help. Keep in mind that nutritional needs vary from person to person, depending on age, sex, health status, and total diet. Information here is intended to help you make informed decisions about your diet, not to substitute for any treatment that may have been prescribed by your physician.

James's case illustrates two very important points: First, that vitamin A overdose can be present with only a few of its typical symptoms—in James's case, those of excessive pressure in the brain. He had none of the other classic symptoms, such as skin changes, fatigue, or nausea.

The other important point bears repeating: A few individuals may succumb to vitamin A overdose at levels far below those needed to cause symptoms in most people. James was taking 10,000 to 20,000 I.U. per day—the lowest dose I have found to be associated with signs of overdose in people age 18 or older. The vast majority of cases on record involve larger doses—often many times larger. Perhaps James's diet provided an unusual level of vitamin A, so that the additional 10,000 to 20,000 I.U. from his supplement brought his total intake into the danger zone. But if not, James was simply highly sensitive to vitamin A. The possibility that a few will overdose on amounts that will not harm most always will exist.

Let's look now at more typical cases of vitamin A overdose in adults.

Recommended Dietary Allowances for Vitamin A

Group/Age	I.U.	R.E.*
Infants 0–6 mon.	1,400	420
Infants 6–12 mon.	2,000	400
Children 1–3 yr.	2,000	400
Children 4–6 yr.	2,500	500
Children 7–10 yr.	3,300	700
Males 11–51+ yr.	5,000	1,000
Females 11–51+ yr.	4,000	800
Pregnant and nursing women	5,000	1,000

Note: The U.S. RDA for vitamin A is 5,000 I.U.

*Retinol Equivalents, another way of measuring vitamin A.

will be well tolerated by many. While 200 to 400 mg. will probably cause no problems for some, I feel that such levels call for regular follow-up by your doctor.

At 500 mg. and higher, of course, there is a risk of toxicity to some people, and a very high risk at doses in the thousands of milligrams. Keep in mind that the risk is not just of a medical problem, but of one that may disable severely and require many months for recovery.

It is precisely this long recovery period that concerns me greatly. None of the other vitamins that we have talked about so far have been linked to symptoms that take so long to subside. There's also the fact that permanent damage has not been completely ruled out in some of the victims who had been taking 2,000 mg. or more.

Recommended Dietary Allowances for Vitamin B_6

Group/Age	Mg.
Infants 0–6 mon.	0.3
Infants 6–12 mon.	0.6
Children 1–3 yr.	0.9
Children 4–6 yr.	1.3
Children 7–10 yr.	1.6
Males 11–14 yr.	1.8
Males 15–18 yr.	2.0
Males 19–50 yr.	2.2
Males 51+ yr.	2.2
Females 11–14 yr.	1.8
Females 15–50 yr.	2.0
Females 51+ yr.	2.0
Pregnant women	2.4–2.6
Nursing women	2.3–2.5

Note: The U.S. RDA for vitamin B_6 is 2 mg.

Recommended Dietary Allowances for Vitamin C

Listed below are the RDA for vitamin C set by the National Academy of Sciences' Food and Nutrition Board. As you may know, many scoff at these allowances, which are based on traditional roles such as preventing the deficiency disease scurvy. Newer roles for vitamin C—such as its role in cancer prevention—have yet to be considered in formulating the allowances.

Because I am convinced that vitamin C plays an important role in cancer prevention, I consider these allowances too low. Yet in 1985, scientists on the RDA committee sought to lower them further on grounds that the RDA should only reflect levels needed to prevent nutritional deficiency diseases. An uproar broke out, and the president of the National Academy of Sciences chose not to release their proposed report. He instead formed a new committee charged with applying broader issues in setting the recommended allowances.

Whether this new group will raise the allowances in

iron a problem nutrient in the United States, mostly for women of childbearing age who lose iron each month during menstruation. Adding to the concern was the fact that some of these women also consume diets notoriously low in iron. As a result, vitamin manufacturers began offering multiple vitamins that also contain iron, and supplements containing only iron became widely available.

Putting a nutrient into the body is one thing, but having it absorbed is another. That is where vitamin C lends a hand. It enhances iron absorption—an important accomplishment for two reasons. First, the body absorbs only a small fraction of the iron taken in. Second, despite nutritionists' best efforts in trying to sell us on the importance of the mineral, some people still take in too little iron. So it only makes sense then that the next best thing to an adequate iron intake is something to enhance absorption of whatever iron is available. However, it's not good sense for all people.

their upcoming RDA report, one can only guess. I do think, however, that we will see a major change in attitudes about vitamin C within a decade. Meanwhile, surveys have found, as have I, that many people are choosing to exceed the RDA in their supplement program.

Group/Age	Mg.
Infants 0–12 mon.	35
Children 1–10 yr.	45
Males 11–14 yr.	50
Males 15+ yr.	60
Females 11–14 yr.	50
Females 15+ yr.	60
Pregnant women	80
Nursing women	100

Note: The U.S. RDA for vitamin C is 60 mg.

If nutrition has a single case where one man's meat is another man's poison, this is it. While most of us can put vitamin C's iron-enhancing effects to good use, some people need to avoid it. They already have too much iron in their bodies, an excess that can cause serious health problems and even death. Their condition is called hemochromatosis, or simply iron overload, and it runs in the family.

Needless to say, if you have hemochromatosis, you should be avoiding vitamin C supplements unless prescribed by your doctor. You probably have been told as much already. In fact, if one of your relatives has hemochromatosis, you should be tested before taking high doses of vitamin C. An odd bronze pigment or discoloration of the skin is a common symptom, and cirrhosis of the liver and disease of the heart muscle are sometimes found upon physical examination if the condition has progressed.

The RDA and Vitamin E: Clearing the Confusion

If you are taking vitamin E supplements, you are familiar with the term "International Unit" or I.U. as a measure of supplement potency. I think of vitamin E in those terms, too.

But the official RDA for vitamin E are issued in a different language. Rather than use the familiar I.U., the scientists in charge use mg. The reasoning is complex.

Even more complex, though, is converting mg. of vitamin E to I.U. That's because vitamin E comes in many forms that can differ in the amount of I.U. per mg. More complications.

If you think of vitamin E in terms of one common form found in supplements, d l-alpha-tocopherol acetate, your worries are over. A mg. of vitamin E is basically equivalent to an I.U. of that, so you can simply think of the numbers below in I.U. instead of mg.

For the trivia seeker, here is how some other common forms of vitamin E translate from mg. to I.U.:

- Synthetic free d l-alpha-tocopherol (not acetate) has 1.1 I.U. per mg.
- Naturally occurring alpha-tocopherol and d-alpha-tocopherol have 1.49 I.U. per mg.

levels of a substance called creatine kinase in the blood. They also had excessive levels of creatinine in the urine. Creatinine, which is made from creatine, is an end product of muscle metabolism. Coupled with the complaints of fatigue and weakness, Dr. Briggs suspected his findings meant the beginning of "some degree of damage to skeletal muscles." Fortunately, these laboratory tests returned to normal within a week after stopping supplemental vitamin E.

Six of Dr. Briggs's participants, however, remained normal throughout the study. They had no complaints while on vitamin E and a wide range of laboratory tests yielded normal readings for them. One can only conclude that sensitivity to vitamin E varies.

• Alpha-tocopherol acetate or d-alpha-tocopherol acetate have 1.36 I.U. per mg.

Now for those RDAs. As you can see, they pale in comparison to the vitamin E supplements we commonly take. And of course, the role of vitamin E in cancer prevention was not considered in formulating the allowances in 1980.

Group/Age	Mg.
Infants 0–6 mon.	3
Infants 6–12 mon.	4
Children 1–3 yr.	5
Children 4–6 yr.	6
Children 7–10 yr.	7
Males 11–14 yr.	8
Males 15+ yr.	10
Females 11+ yr.	8
Pregnant women	10
Nursing women	11

Note: The U.S. RDA for vitamin E is 30 I.U.

NO FATIGUE HERE

You have heard the advocates of the fatigue theory. Now let's give two of the skeptics a turn.

Samuel Ayres, Jr., M.D., and his partner Richard Mihan, M.D., who have pioneered the successful use of vitamin E in troublesome skin disorders, replied, "We have administered therapeutic doses of vitamin E in the range of 400 to 1,600 I.U. daily to hundreds of patients, including ourselves and several members of our office staff, without observing a single case of muscular weakness or fatigue."

W. M. Toone, M.D., of Victoria's Veterans' Hospital, who also

erwise normal. Niacin therapy was discontinued, and during the next two weeks the jaundice deepened.

The patient was transferred to a general hospital. After another ten days of increasing jaundice, further recovery was uneventful, with gradual disappearance of jaundice during the next several months.

A. Arthur Sugerman, M.D., and Charles G. Clark, M.D., the doctors who treated this man, underscored that his jaundice began to lessen within a month of stopping the niacin. But they conclude, "This case was alarming, however, in that the jaundice became more severe in the three weeks following discontinuation

Recommended Dietary Allowances for Niacin

Group/Age	Mg.*
Infants 0–6 mon.	6
Infants 6–12 mon.	8
Children 1–3 yr.	9
Children 4–6 yr.	11
Children 7–10 yr.	16
Males 11–18 yr.	18
Males 19–22 yr.	19
Males 23–50 yr.	18
Males 51+ yr.	16
Females 11–14 yr.	15
Females 15–22 yr.	14
Females 23–50 yr.	13
Females 51+ yr.	13
Pregnant women	15–16
Nursing women	18–19

Note: The U.S. RDA for niacin is 20 mg.

*60 mg. of the amino acid tryptophan can be substituted for 1 mg. of niacin.

"Safe and Adequate" Intakes for Selenium

Until 1980, a nutrient either had an RDA or it didn't. Many minerals had attained status as essential nutrients, but the Committee on Dietary Allowances insisted that information was insufficient to set RDAs for them.

Eventually, a compromise emerged: the "safe and adequate" range. In theory, or according to the committee, these ranges fully meet our needs without exceeding safe limits. It sounds as though the recommendations have been based on research designed to determine optimal intakes.

In some cases, I believe this to be so. The sodium range, for instance, clearly rests on research into the health effects of sodium. I cannot convince myself that the selenium range—50–200 mcg. for adults—was developed in a similar way. It is obvious that many individuals can tolerate intakes greater than 200 mcg. (and also likely that higher intakes may be beneficial). I believe that the "safe and adequate" range represents little but the current range of American intakes expressed in round numbers.

That's my opinion, of course. Most nutritionists still toe the 200 mcg. maximum line. However, I doubt that this limit is based on hard facts. At any rate, here are the "safe and adequate" ranges set by the Committee on Dietary Allowances in 1980.

Group/Age	Mcg.
Infants 0–6 mon.	10–40
Infants 6–12 mon.	20–60
Children 1–3 yr.	20–80
Children 4–6 yr.	30–120
Children 7–10 yr.	50–200
Males 11+ yr.	50–200
Females 11+ yr.	50–200

Recommended Dietary Allowances for Zinc

Group/Age	Mg.
Infants 0–6 mon.	3
Infants 6–12 mon.	5
Children 1–10 yr.	10
Males 11+ yr.	15
Females 11+ yr.	15
Pregnant women	20
Nursing women	25

Note: The U.S. RDA for zinc, designed solely for nutrition labeling purposes, is 15 mg.

declining copper nutrition. It was obvious that high intakes of zinc could inhibit copper absorption in healthy people, too.

Meanwhile, another storm was approaching. It was one that would require decades—not just a year or two—to do its damage. But it was not one to be taken lightly, for at stake was America's number one killer: heart disease.

MORE SUBTLE TROUBLE

I am sure you remember the headlines of a decade ago heralding the discovery of "good cholesterol" in the blood. Who coined the term "good cholesterol," I don't know, but given its real name—high-density lipoprotein-cholesterol—I can see why. In research circles, the term HDL-cholesterol was adopted for simplicity, and study after study found those who had higher levels of it were less likely to succumb to heart disease.

Overjoyed to find a protective factor rather than a harmful one, researchers sought for ways to increase its presence. In the process, they also discovered factors that worked against HDL-cholesterol, causing blood levels to fall. Among the villains was a high intake of supplemental zinc.

Recommended Dietary Allowances for Iron

Group/Age	Mg.
Infants 0–6 mon.	10
Infants 6–12 mon.	15
Children 1–3 yr.	15
Children 4–10 yr.	10
Males 11–18 yr.	18
Males 19+ yr.	10
Females 11–50 yr.	18
Females 51+ yr.	10
Pregnant and nursing women	30–60*

Note: The U.S. RDA for iron is 18 mg. The U.S. RDA is designed solely for nutrition labeling purposes. If you are an adult male or a woman older than 50, your allowance is just a little over half of the U.S. RDA. Therefore, a serving of food, a meal, or a daily diet that contains 50 percent of the U.S. RDA almost meets the recommended allowance for your sex and age group.

*According to the Committee on Dietary Allowances, "The increased requirement [for iron] during pregnancy cannot be met by the iron content of habitual American diets nor by the existing iron stores of many women; therefore, the use of 30–60 mg. of supplemental iron is recommended." The Committee found that iron needs during nursing do not differ much from the needs of nonpregnant women, but recommends continued supplementation of mothers for two to three months after giving birth. This measure is favored to replace any losses in iron stores that may have occurred during the pregnancy.

in treatment will accomplish much less if medical attention is delayed too long.

Even when treatment fully succeeds, however, the experience is a traumatic one. Clearly, the ultimate success is prevention. This goal can be realized simply by keeping iron supplements out of children's reach. If you no longer take the iron tablets prescribed during your pregnancy, consider discarding them.

"Safe and Adequate" Intakes for Copper

Although there are no RDA for copper, the Committee on Dietary Allowances of the National Research Council set these "safe and adequate" allowances in 1980.

Group/Age	Mg.
Infants 0–6 mon.	0.5–0.7
Infants 6–12 mon.	0.7–1.0
Children 1–3 yr.	1.0–1.5
Children 4–6 yr.	1.5–2.0
Children 7–10 yr.	2.0–2.5
Males 11+ yr.	2.0–3.0
Females 11+ yr.	2.0–3.0

Note: There is no U.S. RDA for copper.

milligrams (mg.) daily. Higher-potency supplements are probably not marketed because of serious reactions that occurred years ago when copper sulfate was used medically to induce vomiting.

As for safe amounts, the Committee on Dietary Allowances states that, "It can be assumed that an occasional intake of up to 10 mg. is safe for human adults. However, in order to include an extra margin of safety, it is recommended that the copper intake in adults over extended periods of time be in the range of 2–3 mg./day."

Since 1966, most American diets have provided less than 1.3 mg. of copper daily. Though combining the 3 mg. provided by some supplements with such a dietary intake would exceed the limit set by the RDA committee, such a total intake is unlikely to be unsafe. As acknowledged by the committee, a group of experts convened by the Food and Agriculture Organization/World Health Organization came up with a very different estimate. In 1971, this group calculated upper limits to be about 0.23 mg. per pound of body weight. In other words, this estimate puts the tolerable limit for a 100-pound individual at 23 mg. I am not comfortable with an intake this high, however.

generally is a safe mineral as long as the kidneys are working up to par. That is reassuring indeed. On the other hand, the effectiveness of these organs can decline with age, without our realizing it. This makes me wary of putting the stamp of approval on unlimited doses of magnesium.

Second, as best as I can determine, there are no cases on record of magnesium toxicity caused by a supplement. That makes it almost impossible to set an upper limit for safety, for it would not derive from any experience with magnesium supplements. The only facts to go on come from the antacids containing magnesium, and even here there is far too little experience for sound judgment. The lowest level on record as causing harm in an individual with presumably healthy kidneys amounted to about 1,700 mg. daily of extra magnesium. Of course, George's case may have been a fluke. He may have had some hidden kidney problems after all. However, those who use magnesium-containing

Recommended Dietary Allowances for Magnesium

Group/Age	Mg.
Infants 0–6 mon.	50
Infants 6–12 mon.	70
Children 1–3 yr.	150
Children 4–6 yr.	200
Children 7–10 yr.	250
Males 11–14 yr.	350
Males 15–18 yr.	400
Males 19+ yr.	350
Females 11+ yr.	300
Pregnant and nursing women	450

Note: The U.S. RDA for magnesium is 400 mg.

the disease and his pains relented after he stopped taking the supplements.

- Poor absorption of chromium may account for the low potential for toxicity. Average absorption is estimated to be no more than 2 percent of intake.
- While no toxic effects are known from safe forms of chromium, lack of experience with high doses may account for the clean record. The safest approach is to limit chromium intake to 200 micrograms (mcg.) daily—a level that has been tested without signs of toxicity. The Committee on Dietary Allowances notes that "the safety of 200 mcg. has been established in long-term supplementation trials in human subjects receiving 150 mcg./day in addition to the dietary intake."

In making this conclusion, the committee obviously assumes that the diet provides no more than 50 mcg. This is a fair assumption for the average diet, though some will provide more. Nonetheless, the 150 mcg. supplement is likely to be safe for a healthy adult.

Chromium—"Safe and Adequate"

Although there are no RDA for chromium, the Committee on Dietary Allowances of the National Research Council set these "safe and adequate" allowances in 1980.

Group/Age	Mcg.
Infants 0–6 mon.	10–40
Infants 7–12 mon.	20–60
Children 1–3 yr.	20–80
Children 4–6 yr.	30–120
Children 7–10 yr.	50–200
Males 11+ yr.	50–200
Females 11+ yr.	50–200

Note: There is no U.S. RDA for chromium.

Handbook of Vitamins, Minerals and Hormones

SECOND EDITION

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VNR VAN NOSTRAND REINHOLD COMPANY
NEW YORK CINCINNATI ATLANTA DALLAS SAN FRANCISCO
LONDON TORONTO MELBOURNE

Important groups for activity
β-ionone ring
trans-methyl
Alcoholic hydroxyl

4. **Commercial Production** 18, 22, 29, 32, 34, 35
Chemical—Extraction of fish liver
Synthetic—From citral or β-ionone

5. **Isolation** 9, 17, 22, 29, 32, 34, 35
Sources—Fish liver oils
Method—Saponification in alcoholic KOH. Extract with ether, crystallize

6. **Determination** 14, 16, 27a, 33
Bioassay—Growth rate of rats
Physicochemical—Spectrophotometric determination of blue color on reacting with antimony trichloride or trifluoracetic acid
Radioimmunoassay of retinol-binding protein

DISTRIBUTION AND SOURCES

1. **Occurrence** 16, 19, 22, 25, 26, 29, 32, 34, 35
Plants
Fruit—Provitamin carotenoids—apricots, yellow melons, peaches, prunes
Vegetables—Provitamin carotenoids—beet greens, broccoli greens, carrots, endive, kale, lettuce, mint, mustard, parsley, pumpkins, spinach, sweet potatoes, turnip greens, cress
Nuts—Provitamin carotenoids—in small quantity in most nuts
Animals
Vitamin A in all vertebrates, and carotenoids in certain invertebrates (crustaceans) (A_2 especially in freshwater fish)
Location: Liver, heart, lungs, fat, adrenals, retina, kidney, milk, blood plasma, egg
Provitamin carotenoids found in many animals depending on diet
Hen's egg carotenoid mainly xanthophyll (inactive analog)
Microorganisms: Provitamin carotenoids in algae, fungi, bacteria. No intestinal synthesis of vitamin A

2. **Dietary Sources (Vitamin A and procarotenoids)** 3, 8, 20, 27, 30, 34, 36
High: 10,000-76,000 I.U./100 g
Liver (beef, pig, sheep, calf, chicken)

Liver oil (cod, halibut, salmon, shark, sperm whale)
Carrots, mint, kohlrabi, parsley, spinach, turnip greens, dandelion greens, palm oil
Medium: 1000-10,000 I.U./100 g
Butter, cheese (except cottage), egg yolk, margarine, dried milk, cream
White fish, eel
Kidneys (beef, pig, sheep), liver (pork)
Mangoes, apricots, yellow melons, peaches, cherries (sour), nectarines
Beet greens, broccoli, endive, kale, mustard, pumpkin, sweet potatoes, watercress, tomatoes, leek greens, chicory, chives, collards, fennel, butterhead and romaine lettuce, squash (acorn, butternut, hubba chard
Low: 100-1000 I.U./100 g
Milk
Herring, salmon, oyster, carp, clams, sardines
Grapes, bananas, berries (black-, goose-, rasp-, boysen-, logan-, blue), sweet cherries, olives, oranges, avocados, prunes, kumquats, pineapples, plums, rhubarb, tangerines, red currants
Summer and zucchini squash, asparagus, beans (except kidney), brussels sprouts, cabbages, leeks, peas, artichokes, corn, cucumbers, lentils (dry), peppers, lettuce, celery, cowpeas, rutabagas, okra
Hazelnuts, peanuts, black walnuts, cashew, pecans, pistachios

MEDICAL AND NUTRITIONAL ROLE

1. **Units** 8, 16, 17, 26, 34
1 I.U. = 0.344 μg vitamin A acetate = 0.3 μg retinol = 0.6 μg β carotene
1 R.E. (retinol equivalent) = 1 μg retinol

2. **Normal Blood Levels** 4, 14, 26, 33, 34
15-65 R.E./100 ml serum
50-215 I.U./100 ml serum

3. **Recommended Allowances** 4, 8, 21, 24, 27, 37
Children—1300-2300 I.U./day (400-700 R.E./day)
Adults— { 3300 I.U./day / 1000 R.E./day } (males); { 2640 I.U./day / 800 R.E./day } (females)
Special—Pregnancy { 3300 I.U./day / 1000 R.E./day }; Lactation { 4000 I.U./day / 1200 R.E./day }
Therapeutic dosage—25,000-50,000 I.U./day

MP–2.5-3.5°C
Crystal form–No data
Salts, esters–Succinate, acetate, phosphate
Important groups for activity
Hydroxyl (alcoholic)
Acet., Alc.–Sol.
Benz., Chl., Eth.–Sol.
Absn. max.–292 mμ (alc.)
Chemical nature: aromatic quinoid, alc.
$\alpha_D^{25} = +0.32°$ (alc.)

4. **Commercial Production** 18, 22, 29, 32, 34, 35
Molecular distillation from vegetable oils, "stripping" of vegetable oils

5. **Isolation** 9, 17, 22, 29, 32, 34, 35
Sources–Wheat germ oil, soybean oil, rice oil
Method
Saponify oil with methanolic KOH
Nonsaponifiable fraction has vitamin E, dissolve in ether
Remove sterols with digitonin precipitation
Remove xanthophylls with methanol extraction
Convert tocopherols to allophanate esters with cyanic acid
Crystallize allophanates, hydrolyze, extract vitamin E with ether

6. **Determination** 14, 16, 27a, 33
Bioassay
Rats–Prevent fetal resorption and RBC hemolysis
Chick–Liver storage
Physicochemical–Colorimetric two-dimensional paper chromatography; thin layer chromatography; fluorimetry

DISTRIBUTION AND SOURCES

1. **Occurrence** 16, 19, 22, 25, 26, 29, 32, 34, 35
Plants
Fruits–Apples, olives
Vegetables–Legumes, lettuce, spinach, corn, soybean (oil), mustard, cauliflower, green peppers, turnip greens, kale, kohlrabi, sweet potatoes
Nuts and seeds–Coconuts, peanuts, palm (oil), cottonseed
Grains–Cereals, oils (rice, wheat), oats, brown rice, wheat germ, barley, rye
Animals
Birds–Eggs
Mammals–Liver, fat, muscle, milk, pituitary, adrenals, testes
Microorganisms–Yeast

2. **Dietary Sources** 3, 8, 20, 27, 30, 34, 36
High: 50-300 mg/100 g
Oils, crude–(cottonseed, corn soybean, safflower, wheat germ, sunflower, sesame, castor)
Margarine, mayonnaise, sunflower seeds
Medium: 5-50 mg/100 g
Oils, crude–(coconut, peanut, olive, codliver, palm, walnut)
Wheat germ, apple seeds, alfalfa, barley, dry soybeans, lima beans, poppy and sesame seeds
Chocolate, rose hips, cocoa butter, peanut butter, mint, corn, sweet potatoes
Almonds, brazils, chestnuts, filberts, pecans, walnuts, peanuts
Low: 0.5-5 mg/100 g
Brussels sprouts, carrots, parsnips, mustard, corn, brown rice, lettuce, cauliflower, peas, asparagus, turnip greens, kale, kohlrabi, green peppers, spinach, cabbage
Bacon, beef, lamb, pork, veal, beef liver
Eggs, butter, cheese
Whole wheat flour, dried navy beans, corn meal, oatmeal, coconut, rye, oats, wheat
Blackberries, pears, apples, olives
Cashews, yeast

MEDICAL AND NUTRITIONAL ROLE

1. **Units** 8, 16, 17, 26, 34
1 mg *d*-α-tocopherol = 1.49 I.U.
1 mg *dl*-α-tocopherol acetate = 1 I.U.

2. **Normal Blood Levels (Man)** 4, 14, 26, 33, 34
0.8-1.1 mg/100 ml (serum); 0.22-0.24 mg/100 ml RBC

3. **Recommended Allowances** 4, 8, 21, 24, 27, 37
Children–5-7 I.U./day
Adults–8 I.U./day (females); 10 I.U./day (males)
Special–Related to unsaturated fatty acid intake: increased requirements in pregnancy and lactation, detoxification, aging, stress
Therapeutic dose–5-30 mg/day

4. **Administration** 8, 9, 31, 32, 35
Injection–Used for large dosages
Topical–No data
Oral–Preferred route

Crystal form—Monocl. plates
Salts—Mononitrate, noble metals
Important groups for activity
—OH of $-CH_2CH_2OH$
C-2 of pyrimidine
C-2 of thiazole

Absn. max.—235, 267 mμ
Chemical nature—Base, alc., substituted pyrimidine
Miscellaneous—Charact. odor; pK_a = 4.8, 9.2
α_D = 0 (inactive)

4. **Commercial Production** 18, 22, 29, 32, 34, 35
Synthesis
Pyrimidine + thiazole nuclei synthesized separately and then condensed
Build on pyrimidine with acetamidine

5. **Isolation** 9, 17, 22, 29, 32, 34, 35
Sources—Rice bran, wheat germ, yeast
Method—Aqueous extraction, adsorption on Fuller's earth; elute with quinine sulfate, precipitate as phosphotungstate, decompose precipitate and reprecipitate with $AuCl_3$, extract with water, precipitate from EtOH as hydrochloride

6. **Determination** 14, 16, 27a, 33
Bioassay—Yeast fermentation; polyneuritic rat—rate of cure; bacterial metabolism
Physicochemical—thiochrome fluorescence; polarographic; chromatographic; absorption at 235-267 mμ in neutral solution; at 247 in acid solution

DISTRIBUTION AND SOURCES

Occurrence 16, 19, 22, 25, 26, 29, 32, 34, 35
Plants
Fruit—All, low (except gooseberries, plums, which are medium)
Vegetables—All, low (except beans, green leafy types, cauliflower, corn, peas, potatoes—medium)
Nuts—All, medium (except coconut, which is low)
Grains—All, medium (except outer grain kernels, bran, polishings, wheat germ, which are high)
Animals: All—medium (except pork, which is high, and some fish, which are low)
Microorganisms: Yeast (killed)—high; intestinal bacteria not available
Miscellaneous—Mushrooms—medium

2. **Dietary Sources** 3, 8, 20, 27, 30, 34, 36
High: 1000-10,000 μg/100 g
Wheat germ, rice bran, soybean flour
Yeast
Ham
Medium: 100-1000 μg/100 g
Gooseberries, plums, prunes (dry), raisins (dry), asparagus, beans (kidney, lima, snap, soy, wax), beet greens, broccoli, brussels sprouts, cauliflower, chicory, endive, corn, dandelion greens, kale, kohlrabi, leeks, lentils (dry), parsley, peas, potatoes, watercress, barley, oats, rice (brown), almonds, brazil, cashews, chestnuts, hazelnuts, peanut, pecans, walnuts
Beef, calf, chicken, pork, lamb, turkey meat and organs, mushrooms
Eggs, milk, carp, clams, cod, lobster, mackerel, oysters, salmon
Low: 10-100 μg/100 g
Apples, apricots, avocados, bananas, berries (black-, blue-, cran-, rasp-, straw-), melons (cantaloupe, water, honeydew), cherries, currants, dates (dry), figs, grapes, grapefruit, lemons, oranges, peaches, pears, pineapples, prunes, tangerines
Artichokes, beets, cabbage, carrots, celery, cucumbers, eggplant, lettuce, onions, parsnips, peppers, pumpkins, radishes, rhubarb, spinach, sweet potatoes, turnips, coconut, cheeses, flounder
Haddock, halibut, herring, pike, sardines, scallops, shrimp, trout, tuna

MEDICAL AND NUTRITIONAL ROLE

1. **Units** 8, 16, 17, 26, 34
1 USP unit = 3 μg thiamine HCl = 1 I.U.

2. **Normal Blood Levels (Man)** 4, 14, 26, 33, 34
0.5-1.3 μg/100 ml, serum; 3.5-11.5 g/100 ml, blood

3. **Recommended Allowances** 4, 8, 21, 24, 27, 37
Children—0.7-1.2 mg/day
Adults—1.0-1.1 mg/day, female; 1.2-1.5 mg/day, male
Special—Increased requirements in pregnancy and lactation. Depends on body weight, calorie intake, intestinal synthesis and absorption, fat content of diet (increased pyruvate)
Therapeutic dose—5-30 mg/day

4. **Administration** 8, 9, 31, 32, 35
Injection—Intravenous, intraperitoneal

Important groups
$-CH_2OH$
$-N=$

Chemical nature
Hydroxylated weak nitrogen base, substituted pyridine
$pK_a = 5.0, 8.9$
$\alpha_D = 0$ (inactive)

4. Commercial Production 18, 22, 29, 32, 34, 35
Commercially available as pyridoxine hydrochloride
Synthesized by method of Harris and Folkers; ethoxy acetylacetone condensed with cyanoacetamide
Easiest route for synthesis is probably from oxazoles

Isolation 9, 17, 22, 29, 32, 34, 35
Sources—Rice polishings or bran and yeast
Methods
Adsorb on Fuller's earth or charcoal
Elute with $Ba(OH)_2$
Precipitate impurities with heavy metals
Precipitate with phosphotungstic acid

6. Determination 14, 16, 27a, 33
Bioassay—Animal
Rat acrodynia test
Rat growth and chicken growth assays
Tryptophan loading test
Blood cell
Bioassay—Microbial—Microbioassay
Physicochemical—Photofluorometric procedure detects 4-pyridoxic acid (major metabolite) in urine; chromatographic procedure to detect 4-pyridoxic acid

DISTRIBUTION AND SOURCES

1. Occurrence 16, 19, 22, 25, 26, 29, 32, 34, 35
Plants
Fruit—All low, except bananas, avocados, grapes, pears (medium)
Vegetables—All low or medium
Nuts—All high
Miscellaneous—Cereals—medium, except brown rice, wheat germ (high); and blackstrap molasses (high)
Animals: All medium, except herring, salmon, liver (high)
Microorganisms: All high or medium—yeast, intestinal bacteria (high); some other bacteria

2. Dietary Sources 3, 8, 20, 27, 30, 34, 36
High: 1000-10,000 μg/100 g
Liver (beef, calf, pork), herring, salmon
Walnuts, peanuts, wheat germ, brown rice
Yeast, blackstrap molasses
Medium: 100-1000 μg/100 g
Bananas, avocados, grapes, pears
Barley, cabbage, carrots, corn, oats, peas, potatoes, rye, kale, tomatoes, turnips, yams, brussels sprouts, cauliflower, spinach, soybeans, wheat
Beef, lamb, pork, veal (heart, brains, kidney); cod, flounder, halibut, mackerel, whale, sardines, tuna
Butter, eggs
Low: 10-100 μg/100 g
Apples, cantaloupes, grapefruit, lemons, oranges, peaches, raisins, strawberries, watermelons, cherries, currants (red)
Asparagus, beans, beet greens, lettuce, onions
Cheese, milk

MEDICAL AND NUTRITIONAL ROLE

1. Units 8, 16, 26, 34
By weight, mg or μg

2. Normal Blood Levels 4, 14, 26, 33, 34
4.6-7.2 μg/100 ml, serum; 3.1-4.3 μg/100 ml, blood

3. Recommended Allowances 4, 8, 21, 24, 27, 37
Children—0.9-1.6 mg/day*
Adults—2.0 mg/day* (females); 2.2 mg/day* (males)
Special—Pregnancy, 2.6 mg/day; lactation, 2.5 mg/day
Therapeutic Dose—25-100 mg/day

4. Administration 8, 9, 31, 32, 35
Injection—Intravenous, subcutaneous
Topical—No data
Oral—Preferred route

5. Factors Affecting Availability 3, 8, 20, 27, 27a
Decrease
Administration of isoniazid

*Depends on protein content of food and inborn errors of metabolism; irradiation increases need.

Black currant, guava, rose hips
Medium: 50-100 mg/100 g
Beet greens, cabbages, cauliflower, chives, kohlrabi, mustard, watercress, spinach
Lemons, oranges, papayas, strawberries
Low: 25-50 mg/100 g
Asparagus, lima beans, beet greens, chard, cowpeas, mint, okra, spring onions, peas, potatoes, radishes, rutabagas, turnips, dandelion greens, fennel, soybeans, summer squash
Gooseberries, passion fruit, grapefruit, limes, loganberries, mangoes, cantaloupes, honeydews, red currants, white currants, tangerines, raspberries, tomatoes, kumquats

MEDICAL AND NUTRITIONAL ROLE

1. **Units** 8, 16, 17, 26, 34
1 I.U. = 1 U.S.P. unit = 0.05 mg *l*-ascorbic acid

2. **Normal Blood Levels** 4, 14, 26, 33, 34
0.3-1.0 mg/100 ml, serum
0.4-1.5 mg/100 ml, blood } vary with diet
25 mg/100 ml, WBC

3. **Recommended Allowances** 4, 8, 21, 24, 27, 37
Children—45 mg/day
Adults—60 mg/day
Special—Pregnancy (80 mg/day), lactation (100 mg/day); increased with infection, stress, trauma, allergies, old age, increased protein consumption
Therapeutic dose—100-1000 mg/day

4. **Administration** 8, 9, 31, 32, 35
Injection—Intramuscular, intravenous
Topical—No data
Oral—Preferred route

5. **Factors Affecting Availability** 3, 8, 20, 27
Decrease
Damage to adrenal cortex, presence of antagonists
Food preparation (oxidation, storage, leaching, cooking)
Increase
Storage in body (adrenal cortex)
Antioxidants, synergists in diet

6. **Deficiency Symptoms** 3, 4, 16, 21, 24, 27, 27a, 30
General
Hyperkeratotic papules on buttocks and calves
Perifollicular hemorrhage, edema
Wound healing failure
Teeth and gum defects
Weakness, listlessness, rough skin, aching joints
Scorbutic bone formation
Lab animals
Anemia, loss of weight
Abnormal collagen, no intercellular cement

7. **Effects of Overdose, Toxicity** 3, 4, 16, 22, 26, 29, 31, 32, 34
Possible kidney stones, in gouty individuals
Inhibitory in excess doses on cellular level (mitosis inhibited)
Possible damage to β-cells of pancreas and decreased insulin production by dehydroascorbic acid
Possible diarrhea, allergies, reproductive failure, vitamin C dependenc thrombosis, aciduria (oxalic, folic, uric), B_{12} inactivation

METABOLIC ROLE

1. **Biosynthesis** 3, 19, 25, 28
Precursors—*d*-Mannose, *d*-fructose, glycerol, sucrose, *d*-glucose, or D-galactose
Intermediates—UDP glucose, *d*-glucuronic acid, gulonic acid, *l*-gulonolactone, (Mn^{2+} cofactor)

2. **Production—Species and sites** 8, 9, 16, 26, 34
All animals (except primates, guinea pig, fruit bat, late birds, insects, invertebrates, fishes)
Organs: Kidney [reptiles, amphibians, primitive birds (chicken)], liver (mammals, song birds)
Plants (green leaves, fruit skin)
Cell sites—Microsomes, mitochondria, golgi

3. **Storage Sites** 8, 14, 29, 31, 32
Adrenal cortex (small amount), liver

4. **Blood Carriers** 8, 14, 29, 31, 32
Free in blood, especially in white blood cells

5. **Half-life** 8, 9, 29, 31, 32, 34
16 days (man), few days (guinea pig)

Beets, beet greens, brussels sprouts, cabbage, carrots, cauliflower, celery, chicory, endive, cucumbers, dandelion greens, eggplant, kohlrabi, lettuce, onions, parsnips, peppers, pumpkins, radishes, rhubarb, spinach, sweet potatoes, tomatoes, turnips, watercress
Coconuts, pecans
Eggs, milk

MEDICAL AND NUTRITIONAL ROLE

1. **Units** 8, 16, 17, 26, 34
   By weight, mg equivalents

2. **Normal Blood Levels** 4, 14, 26, 33, 34
   .05 mg/100 ml, serum; 3.0 mg/100 ml, blood

3. **Recommended Allowances** 4, 8, 21, 24, 27, 37
   Children—9-16 mg equivalents/day*
   Adults—16-19 mg equivalents/day—male; 13-15 mg equivalents/day—female*
   Special—Pregnancy, 15 mg equivalents/day; lactation, 20 mg equivalents/day
   Therapeutic dose—100-1000 mg/day (niacinamide)

4. **Administration** 8, 9, 31, 32, 35
   Injection—I.V.
   Topical—No data
   Oral—Preferred route

5. **Factors Affecting Availability** 3, 8, 20, 27, 27a
   Decrease
   Cooking losses
   Bound form in corn, greens, seeds, partially unavailable
   Oral antibiotics
   Decreased absorption—Disease
   Decreased tryptophan converted in B_6 deficiency
   Increase
   Alkali treatment of cereals
   Storage in liver, possibly muscle, kidney
   Increased intestinal synthesis

6. **Deficiency Symptoms** 3, 4, 16, 21, 24, 27, 27a, 30
   General (man)—Pellagra
   Retarded growth, achlorhydria

*Depends on tryptophan content of diet; allow 10 mg equivalents for each 600 mg dietary tryptophan, assume 60 g/day protein in diet has 600 mg tryptophan.

Weakness, anorexia, indigestion, lassitude, dermatitis, pigmentation, diarrhea, tongue erythema, irritability, headaches, insomnia, memory loss
Histological changes in central nervous system (dog, cat—blacktongue)
Drooling (dog, cat—blacktongue)
Perosis (chickens)
Poor feathering (chickens)

7. **Effects of Overdose, Toxicity** 3, 4, 16, 22, 26, 29, 31, 32, 34
   Man—Limited toxicity, starting approx. 1-4 g/kg dosage with individual variations in sensitivity—Burning, itching skin; peripheral vasodilation; decreased serum cholesterol, fatty liver; stimulated central nervous system; increased: pulse rate, respiratory rate, cerebral blood flow; decreased blood pressure
   Rat—Respiratory paralysis, ketosis
   Dogs—Death
   Chick—Inhibition of growth, fatty liver

METABOLIC ROLE

1. **Biosynthesis** 3, 19, 25, 28
   Precursors—Tryptophan (animals, bacteria). Glycerol and succinic acid (plants)
   Intermediates—Kynurenine, hydroxyanthranilic acid, quinolinic acid

2. **Production: Species and sites** 3, 19, 25, 28
   Fungi—*Neurospora*
   Plants—Leaves, germinating seeds, shoots
   Bacteria—Intestinal
   Animals—Tissues (not intestinal)

3. **Storage** 8, 9, 16, 26, 34
   Liver, heart, muscle

4. **Blood Carriers** 8, 14, 29, 31, 32
   Mostly as DPN in blood corpuscles

5. **Half-life** 8, 9, 29, 31, 32, 34
   1/3 of intake excreted in 24 hr

6. **Target Tissues** 14, 16, 29, 31, 32, 34
   Liver (storage), heart, muscle, kidney, skin, gastrointestinal tract, spinal cord

THIRTEENTH EDITION

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TABLE 11-2. RECOMMENDED DAILY
FOOD AND NUTRITION BOARD, NATIONAL ACADEMY

	AGE (yr)	WEIGHT kg (lb)	HEIGHT cm (in.)	ENERGY (kcal)†	PROTEIN (Gm)	FAT-SOLUBLE VITAMINS			
						VITAMIN A ACTIVITY (RE)‡	VITAMIN A ACTIVITY (IU)	VITAMIN D (IU)	VITAMIN E ACTIVITY** (IU)
Infants	0.0-0.5	6 (14)	60 (24)	kg × 117	kg × 2.2	420§	1400	400	4
	0.5-1.0	9 (20)	71 (28)	kg × 108	kg × 2.0	400	2000	400	5
Children	1-3	13 (28)	86 (34)	1300	23	400	2000	400	7
	4-6	20 (44)	110 (44)	1800	30	500	2500	400	9
	7-10	30 (66)	135 (54)	2400	36	700	3300	400	10
Males	11-14	44 (97)	158 (63)	2800	44	1000	5000	100	12
	15-18	61 (134)	172 (69)	3000	54	1000	5000	400	15
	19-22	67 (147)	172 (69)	3000	54	1000	5000	400	15
	23-50	70 (154)	172 (69)	2700	56	1000	5000		15
	51+	70 (154)	172 (69)	2400	56	1000	5000		15
Females	11-14	44 (97)	155 (62)	2400	44	800	4000	400	12
	15-18	54 (119)	162 (65)	2100	48	800	4000	400	12
	19-22	58 (128)	162 (65)	2100	46	800	4000	400	12
	23-50	58 (128)	162 (65)	2000	46	800	4000		12
	51+	58 (128)	162 (65)	1800	46	800	4000		12
Pregnant				+300	+30	1000	5000	400	15
Lactating				+500	+20	1200	6000	400	15

	WATER-SOLUBLE VITAMINS							MINERALS					
	ASCORBIC ACID (mg)	FOLACIN†† (μg)	NIACIN‡‡ (mg)	RIBOFLAVIN (mg)	THIAMINE (mg)	VITAMIN B_6 (mg)	VITAMIN B_{12} (μg)	CALCIUM (mg)	PHOSPHORUS (mg)	IODINE (μg)	IRON (mg)	MAGNESIUM (mg)	ZINC (mg)
Infants	35	50	5	0.4	0.3	0.3	0.3	360	240	35	10	60	3
	35	50	8	0.6	0.5	0.4	0.3	540	400	45	15	70	5
Children	40	100	9	0.8	0.7	0.6	1.0	800	800	60	15	150	10
	40	200	12	1.1	0.9	0.9	1.5	800	800	80	10	200	10
	40	300	16	1.2	1.2	1.2	2.0	800	800	110	10	250	10
Males	45	400	18	1.5	1.4	1.6	3.0	1200	1200	130	18	350	15
	45	400	20	1.8	1.5	2.0	3.0	1200	1200	150	18	400	15
	45	400	20	1.8	1.5	2.0	3.0	800	800	140	10	350	15
	45	400	18	1.6	1.4	2.0	3.0	800	800	130	10	350	15
	45	400	16	1.5	1.2	2.0	3.0	800	800	110	10	350	15
Females	45	400	16	1.3	1.2	1.6	3.0	1200	1200	115	18	300	15
	45	400	14	1.4	1.1	2.0	3.0	1200	1200	115	18	300	15
	45	400	14	1.4	1.1	2.0	3.0	800	800	100	18	300	15
	45	400	13	1.2	1.0	2.0	3.0	800	800	100	18	300	15
	45	400	12	1.1	1.0	2.0	3.0	800	800	80	10	300	15
Pregnant	60	800	+2	+0.3	+0.3	2.5	4.0	1200	1200	125	18+§§	450	20
Lactating	80	600	+4	+0.5	+0.3	2.5	4.0	1200	1200	150	18	450	25

* The allowances are intended to provide for individual variations among most normal persons as they live in the USA under usual environmental stresses. Diets should be based on a variety of common foods in order to provide other nutrients for which human requirements have been less well defined.

† Kilojoules (kJ) = 4.2 × kcal.

‡ Retinol equivalents.

§ Assumed to be all as retinol in milk during the first 6 mo of life. All subsequent intakes are assumed to be ½ as retinol and ½ as β-carotene when calculated from international units. As retinol equivalents, ⅓ are as retinol and ⅔ as β-carotene.

** Total vitamin E activity, estimated to be 80% as α-tocopherol and 20% other tocopherols.

†† The folacin allowances refer to dietary sources as determined by *Lactobacillus casei* assay. Pure forms of folacin may be effective in doses less than ¼ of the recommended dietary allowance.

‡‡ Although allowances are expressed as niacin, it is recognized that on the average 1 mg of niacin is derived from each 60 mg of dietary tryptophan.

§§ This increased requirement cannot be met by ordinary diets; therefore, the use of supplemental iron is recommended.

soups, broth, eggs, scraped meat, cream or cottage cheese, gelatin, custard, tapioca, milk, sugars, and jams. Commercially available oral fat emulsions (1 to 2 tbsp 3 or 4 times/day after meals) or nutritional supplements may be used to increase the caloric intake. A daily multivitamin supplement may be needed. (See TABLE 11-5, p. 1127.)

Foods to avoid: Coarse cereals, raw fruits and vegetables, strong cheese, fried food, rich desserts, fish and solid meat or fowl.

High-Protein Diet

Indications: Protein depletion from any cause; preoperative nutritional preparation; compensated hepatic disorders. A high-protein diet is **contraindicated** when hepatic decompensation may lead to hepatic coma or when renal function is so impaired that added protein may result in progressive azotemia and acidosis.

Principle: To ensure an adequate intake of calories, vitamins, minerals, and water, and to increase the protein intake to two or more times that of the normal diet, using protein of high biologic value.

Content: The sample diet in TABLE 11-6 (p. 1128) supplies about 130 to 140 Gm of protein. If additional protein intake is desired, cottage cheese, high-protein drinks, or protein hydrolysates are indicated. The drinks are prepared by adding casein, powdered milk, or egg whites to skim milk or an eggnog, and flavoring the mixture with cocoa and dextrose or maltose to make it palatable. Prepared flavored protein supplements are available commercially.

chelated. Acute toxic reactions to penicillamine (e.g., fever, rash, leukopenia, or thrombocytopenia) occur in about one third of patients, but it is often possible to discontinue the drug temporarily and restart therapy with concomitant administration of corticosteroids. Platelet and WBC counts should be obtained every 1 to 2 wk for several weeks, with monthly counts thereafter. Occasionally, the start of treatment exacerbates neurologic symptoms, but these usually subside with temporary cessation of the drug. Chronic administration rarely is associated with toxic side effects other than easily preventable or correctable vitamin B_6 and iron deficiency. Proteinuria usually does not occur during penicillamine treatment. However, frequent urinalyses should be obtained during therapy as penicillamine may rarely precipitate Goodpasture's syndrome. Although not accepted universally as a mode of therapy in Wilson's disease, prevailing opinion is that lifelong penicillamine therapy is indicated in conjunction with oral copper chelators and a low-copper diet.

OTHER TRACE ELEMENTS

Iodine

Nearly 80% of the total iodine present in the body is found in the thyroid, almost all as **thyroglobulin**, the storage form of thyroid hormone. Marine foods are rich dietary sources. Drinking water, which supplies a relatively small proportion of the intake, reflects the soil content of locally grown foods.

Iodine deficiency results in colloid or endemic goiter (see GOITER in §12, Ch. 3), and should be corrected with iodized salt, not with Lugol's solution.

Zinc

The body contains 1 to 2.5 Gm of zinc, found mainly in bones, teeth, hair (which can be used to assess zinc status), skin, and testes. In the plasma, one third is attached loosely to albumin, and about two thirds is firmly bound to globulins. Plasma levels relate closely to dietary intake, but various diseases may cause low levels. Zinc is also present in RBCs, mainly as carbonic anhydrase, and in WBCs and platelets. For estimated dietary requirements, see TABLE 11-2.

Chelation of dietary zinc by high fiber and phytate content of whole-meal bread, geophagia, and parasitism may be factors leading to reduced absorption and deficiency problems. In one USA study a small proportion of children over age 4 yr had low zinc status, associated with poor appetite, poor growth, and impaired taste (hypogeusia). With zinc treatment appetite improved, taste became normal, and catch-up growth occurred. High milk consumption, poor in zinc, may have been responsible. A syndrome of dwarfism and hypogonadism with low zinc status, seen in the Middle East, has been shown to respond to zinc supplementation.

Acrodermatitis enteropathica, an inherited, usually lethal disorder mediated by malabsorption, has been reported to respond to small zinc supplements (35 mg zinc sulfate daily) with complete clearance of skin lesions and restoration of normal bowel function, but this awaits confirmation.

Chromium

Trivalent chromium increases glucose tolerance and acts as a cofactor with insulin in promoting normal glucose utilization, growth, and longevity in rats and mice. It improves glucose tolerance only in those humans who are chromium-deficient. Glucose tolerance is usually impaired in energy-protein malnutrition, especially in kwashiorkor, and some cases have shown a dramatic response to trivalent chromium.

Although it does not pose a nutritional problem, hexavalent chromium is much more toxic than trivalent chromium. Its extensive use in industry presents a hazard to exposed workers and precautions should be taken in its use.

Cobalt

The significance of cobalt in health and nutrition is confined, as far as is known, to its presence in the cobalamin (vitamin B_{12}) molecule. Dietary deficiency in man has not been proved.

Cobaltous chloride in quite large doses (20 to 30 mg/day) has been advocated in addition to iron in the treatment of iron-deficiency anemia, but should be used only if cobalt deficiency is suspected, as it is potentially toxic. Overdosing in infants may cause thyroid hyperplasia, myxedema, and congestive heart failure.

Selenium

Selenium is involved in the reoxidation of reduced glutathione and has close metabolic interrelationships with vitamin E. It is necessary for growth and fertility in animals, but human deficiency has not been demonstrated even in locales where livestock are deficient. In areas where livestock are affected by selenosis, humans have not been shown to suffer from toxicity, although oral ingestion of selenium salts is highly toxic.

Manganese

Manganese is a component of several enzyme systems and is essential for normal bone structure. Intake varies greatly, depending mainly upon the consumption of rich sources such as unrefined cereals, green leafy vegetables, and tea. Human deficiency has not been reported. Manganese poisoning is usually limited to those who mine and refine ore; prolonged exposure causes neurologic symptoms resembling parkinsonism or Wilson's disease.

Fluorine

Bones and teeth contain most of the body's fluorine. Sea fish and tea are rich sources, but intake is mainly from drinking water. Fluoridation of water sources that contain less than the ideal level of 1 ppm significantly reduces the incidence of caries in the community.

Excess accumulation of fluorine, **fluorosis**, occurs in teeth and bone in proportion to the level and duration of intake. Fluorosis is most evident in permanent teeth that develop during high fluorine intake. Deciduous teeth are affected only at high levels of intake. The earliest changes, chalky-white, irregularly distributed patches on the surface of the enamel, become infiltrated by yellow or brown staining, giving rise to the characteristic "mottled" appearance. Severe fluorosis weakens the enamel, resulting in surface pitting. Bony changes, characterized by osteosclerosis and exostoses, usually are seen only after prolonged high intake in adults.

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5. OBESITY

The incidence of obesity coincides with the availability of food, obesity being conspicuously absent during famine. It is a serious common condition in affluent cultures chiefly because of the abundance of food and the decreased amount of physical activity.

Etiology

The intake of food in excess of body requirements—overeating—is the principal cause of obesity. The capacity of the body to store protein and carbohydrate is limited, and excess food is converted into fat and stored. All of the metabolic factors that modulate and control the biochemical pathways and the efficiency of energy metabolism are poorly understood. Genetic factors may be involved, since

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In r Application of)
)
JEFFRY W. KREAMER)
)
Serial No. 08/071,052) Group Art Unit 1205
Filed June 4, 1993)
For: ASPIRIN AND VITAMIN) Examiner: T. Criares
AND/OR TRACE ELEMENT)
COMPOSITIONS FOR THE)
PREVENTION AND TREATMENT)
OF VASCULAR DISEASE)

Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132

Dear Sir:

I, Larry H. Hollier, M.D., declare as follows:

1. I received my bachelor of science degree in 1964 from Louisiana State University, and my medical degree in 1968 from Louisiana State University School of Medicine. My internship and residency were at Charity Hospital of Louisiana in New Orleans, Louisiana.

2. I have received certification in general vascular surgery in 1983 and 1991, and have been certified by the American Board of Surgery in 1976 and 1987.

3. I have been a fellow of the American College of Surgeons since 1977 and a fellow in the American College of Cardiology since 1981.

4. I have been a member of th International Society for Cardiovascular Surgery since 1989, and a member of the American Surgical Association since 1990.

5. I have been a member of the American College of Physician Inventors since 1992, and a member of the American Heart Association, Council on Cardiothoracic and Vascular Surgery since 1992.

6. I have published, either alone or in conjunction with others, over 200 articles addressing topics relating to vascular medicine.

7. From 1976 until 1979 I was an Assistant Professor then Associate Professor of Surgery at the Louisiana State University Medical School.

8. From 1980 until 1987 I was an Assistant Professor then Associate Professor of Surgery at the Mayo Medical School.

9. From 1987-1993 I was chairman of the Department of Surgery at the Ochsner Clinic in New Orleans, Louisiana, and on the staff executive committee of the Alton Ochsner Medical Foundation in New Orleans, Louisiana.

Currently, I am chairman of the Department of Surgery and Executive Director of Clinical Affairs at Health Care International (Scotland).

10. I am familiar with the subject matter of the above-identified Patent Application.

11. Multi-vitamins are the most typical type of over-the-counter vitamin supplement taken by the general public.

12. Most leading multi-vitamin tabl ts typically contain the following vitamins: Vitamin A, Vitamin B-6, Vitamin C, Vitamin E and Niacin (typically deliv r d as Niacinamide).

13. Most multi-vitamin tablets contain one or more of th following trace elements: selenium, zinc, iron, copper, cobalt and manganese.

14. The results of the unpublished observational study conducted by the University of Southern California, noted in the above-identified Application, shows unexpectedly beneficial results from the combination of aspirin and vitamins taken by the test subjects.

15. The attached graphic representation of the data collected in the University of Southern California study shows that the anticipated additive effect of weekly administrations of aspirin with vitamins would be a slight increase in deaths, the known benefits of aspirin and vitamins notwithstanding.

16. The results of the University of Southern California study show that the weekly administration of aspirin in combination with vitamins reduces the risk of cardiovascular deaths, myocardial infarction, ischemic heart disease, other heart di - ease, and total deaths more than either the weekly administration of aspirin alone or the administration of vitamins alone.

17. The University of Southern California data shows that the anticipated benefits of combining the weekly combination of aspirin with the administration of vitamins would be detrimental to the health of individuals so treated.

18. The results of the University of Southern California study show that th actual eff ct of we kly administration of aspirin in combination with vitamins is a reduction in the risk of cardiovascular deaths, miocardial infarction, ischemic heart disease, other heart disease, and total deaths beyond the additive affect of the weekly administration of aspirin in combination with vitamins anticipated by the data.

19. Contrary to the anticipated deleterious effects of combining weekly administrations of aspirin along with vitamins, the University of Southern California study shows the actual effect of weekly administration of aspirin with vitamins is unanticipatedly beneficial to individuals so treated.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 3/1/94

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Vitamin C (as Niacinamide Ascorbate)	90 mg.	150
Folic Acid	400 mcg.	100
Vitamin B-1 (as Thiamine Mononitrate)	5 mg.	333
Vitamin B-2 (as Riboflavin)	5 mg.	294
Niacin (as Niacinamide Ascorbate)	30 mg.	150
Vitamin B-6 (as Pyridoxine Hydrochloride)	5 mg.	250
Vitamin B-12 (as Cyanocobalamin)	12 mcg.	200
Vitamin D	400 I.U.	100
Biotin	40 mcg.	13
Pantothenic Acid (as Calcium Pantothenate)	10 mg.	100
Vitamin K-1 (as Phytonadione)	25 mcg.	**
Calcium (as Dicalcium Phosphate)	162 mg.	16
Phosphorus (as Dicalcium Phosphate)	125 mg.	13
Iodine (as Potassium Iodide)	150 mcg.	100
Iron (as Ferrous Fumarate)	25 mg.	139
Magnesium (as Magnesium Oxide)	100 mg.	25
Copper (as Cupric Oxide)	3 mg.	150
Manganese (as Manganese Sulfate)	7.5 mg.	**
Potassium (as Potassium Chloride)	7.7 mg.	**
Chloride (as Potassium Chloride)	7 mg	**
Zinc (as Zinc Oxide)	15 mg.	100
Chromium (as Chromium Chloride)	50 mcg.	**
Selenium (as Sodium Selenate)	50 mcg.	**
Molybdenum (as Sodium Molybdate)	50 mcg.	**
Tin (as Stannous Chloride)	10 mcg.	**
Vanadium (as Sodium Metavanadate)	10 mcg.	**
Nickel (as Nickelous Sulfate)	5 mcg.	**
Silicon (as Metasilicates and Oxides)	2 mg.	**
Boron (as Borates)	150 mcg.	**

* %U.S. Recommended Daily Allowance for adults and children 4 or more years of age.
** Recognized as essential in human nutrition but no U.S. RDA established.
*** U.S. RDA not established. Partial conversion to Vitamin A occurs in the body, up to a maximum 8000 I.U.

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EACH TABLET CONTAINS:		For Adults–Percentage of U.S. Recommended Daily Allowance (U.S. RDA)
Vitamin A (as Acetate and Beta Carotene)	5000 IU	(100%)
Vitamin D	400 IU	(100%)
Vitamin E	30 IU	(100%)
Vitamin K_1	25 mcg*	
Vitamin C	60 mg	(100%)
Folic Acid	400 mcg	(100%)
Vitamin B_1	1.5 mg	(100%)
Vitamin B_2	1.7 mg	(100%)
Niacinamide	20 mg	(100%)
Vitamin B_6	2 mg	(100%)
Vitamin B_{12}	6 mcg	(100%)
Pantothenic Acid	10 mg	(100%)
Biotin	30 mcg	(10%)
Calcium	162 mg	(16%)
Phosphorus	109 mg	(11%)
Iodine	150 mcg	(100%)
Iron	18 mg	(100%)
Magnesium	100 mg	(25%)
Copper	2 mg	(100%)
Zinc	15 mg	(100%)
Manganese	2.5 mg*	
Potassium	40 mg*	
Chloride	36.3 mg*	
Chromium	25 mcg*	
Molybdenum	25 mcg*	
Selenium	20 mcg*	
Nickel	5 mcg*	
Tin	10 mcg*	
Silicon	2mg*	
Vanadium	10 mcg*	
Boron	150 mcg*	

RECOMMENDED INTAKE:
Adults, 1 tablet daily.
Keep this and all medications out of the reach of children.
Store at Room Temperature.

Inactive Ingredients: FD&C Yellow No. 6, Hydroxypropyl Methylcellulose, Lactose, Magnesium Stearate, Microcrystaline Cellulose, Polysorbate 80, Polyvinylpyrrolidone, Stearic Acid, Titanium Dioxide and Triethyl Citrate
*No U.S. RDA established.

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VITAMINS	QUANTITY	% U.S. RDA	MINERALS	QUANTITY	% U.S. RDA
Vitamin A (as Acetate and Beta Carotene)	5000 I.U.	100	Iron (elemental)	18 mg	100
			Calcium (elemental)	130 mg	13
Vitamin C	60 mg	100	Phosphorus	100 mg	10
Thiamine (B-1)	1.5 mg	100	Iodine	150 mcg	100
Riboflavin (B-2)	1.7 mg	100	Magnesium	100 mg	25
Niacin	20 mg	100	Copper	2 mg	100
Vitamin D	400 I.U.	100	Zinc	15 mg	100
Vitamin E	30 I.U.	100	Chromium	10 mcg	*
Vitamin B-6	2 mg	100	Selenium	10 mcg	*
Folic Acid	0.4 mg	100	Molybdenum	10 mcg	*
Vitamin B-12	6 mcg	100	Manganese	2.5 mg	*
Biotin	30 mcg	10	Potassium	37.5 mg	*
Pantothenic Acid	10 mg	100	Chloride	34 mg	*

*No U.S. RDA established

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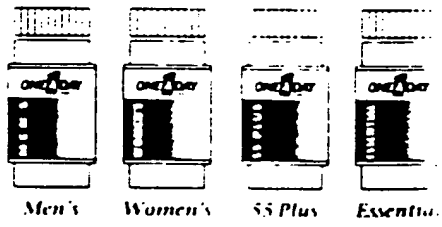

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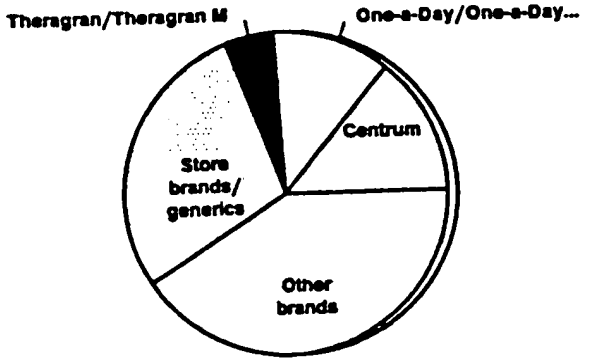
Drugs (SIC 2834)

Vitamin Market - Adult



Leading brands and manufacturers; shares in percent.

Centrum (Lederle)	14.0%
One-a-Day/One-a-Day with Iron (Miles)	12.0
Theragran/Theragran M (Squibb)	5.0
Store brands/generics	28.0
Other brands	41.0

Source: *Drug Topics*, January 8, 1990, p. 46.

★723★
Drugs (SIC 2834)

Vitamin Market - Baby

Leading brands and manufacturers; shares in percent.

Poly-Vi-Sol (Mead Johnson)	57.0%
Flintstones (Miles)	21.0
Tri-Vi-Sol (Mead Johnson)	7.0
Other brands	15.0

Source: *Drug Topics*, January 8, 1990, p. 48.

★724★
Drugs (SIC 2834)

Vitamin Market - Child

Leading brands and manufacturers; shares in percent.

Flintstones (Miles)	34.0%
Store brands/generics	23.0
Poly-Vi-Sol (Mead Johnson)	6.0
Sunkist (Ciba)	5.0
Other brands	32.0

Source: *Drug Topics*, January 8, 1990, p. 48.

★725★
Drugs (SIC 2834)

World Ulcer Drugs - Brands

Shares of the peptic ulcer remedy market in 1989, shown in percent by brand.

Zantac (ranitidine)	44.0%
Tagamet (cimetidine)	22.0
Gaster/Pepcid (famotidine)	8.0
Axid (nizatidine)	2.0
Altat (roxatidine)	<1

Source: Investext, Thomson Financial Networks, January 1, 1990 from Smith New Court Securities PLC.

★726★
Drugs (SIC 2834)

World Ulcer Drugs - Producers

Shares of the peptic ulcer remedy market in 1989, shown in percent.

Glaxco	44.0%
Smithkline Beecham	22.0
Yamanouchi/Merck	8.0
Eli Lilly	2.0
Teikoku Hormone/Hoechst	<1

Source: Investext, Thomson Financial Networks, January 1, 1990 from Smith New Court Securities PLC.

PAPERS

Aspirin use and chronic diseases: a cohort study of the elderly

Annlia Paganini-Hill, Ann Chao, Ronald K Ross, Brian E Henderson

Abstract

Objective—To evaluate the associations between the use of aspirin and the incidences of cardiovascular diseases, cancers, and other chronic diseases.

Design—Postal questionnaire survey to elicit details of aspirin use.

Setting—Californian retirement community.

Subjects—All 22 781 residents of the community (white, affluent, and well educated) were sent a questionnaire that included questions on medical history and the use of drugs such as analgesics, laxatives, and vitamin supplements. In all 61% responded (13 967, 8281 women and 5106 men; median age 73). They formed the cohort that was followed up for 6½ years using discharge summaries from three hospitals serving the area and death certificates from the health department. Only 13 respondents were lost to follow up but seemed not to have died.

Main outcome measures—Incidences of cardiovascular diseases, cancers, gastrointestinal bleeding, ulcers, and cataracts were compared in participants who did and did not take aspirin daily.

Results—Age adjusted incidences were computed with an internal standard and five age groups. By 1 January 1988 there had been 28 incident cases of kidney cancer among all participants; 341 incident cases of stroke, 253 of acute myocardial infarction, 210 of ischaemic heart disease, and 917 of other heart disease were reported among respondents without a reported history of angina, myocardial infarction, or stroke. The incidence of kidney cancer was raised among those who took aspirin daily compared with those who did not take it, although the increase was significant only in men (relative risks=6·3, 95% confidence interval 2·2 to 17, for men and 2·1, 0·53 to 8·5, for women). Those who took aspirin daily showed no increased risk of any other cancer, except colon cancer for both sexes combined (relative risk=1·5, 1·1 to 2·1). The risk of acute myocardial infarction was reduced slightly among regular users of aspirin in men but not women. The risk of ischaemic heart disease was almost doubled in those who took aspirin daily compared with non-users (relative risks=1·9, 1·1 to 3·1, for men and 1·7, 1·1 to 2·7, for women). Small, non-significant increased risks of stroke were observed in both sexes.

Conclusion—The daily use of aspirin increased the risk of kidney cancer and ischaemic heart disease.

Introduction

In the United States vascular diseases account for about 1·2 million deaths, or half of all deaths each year. Recently, the results of a large clinical trial showing that regular use of aspirin may prevent myocardial infarction have received widespread publicity.[1] Given the preliminary nature of this published report, the limitation of the study to men, some evidence of internal inconsistency of the results, and the contradictory findings of other trials,[2] this relation cannot yet be established securely.

As part of a prospective study on lifestyle practices and disease prevention in a retirement community in southern California, we assessed the association between regular use of analgesics containing aspirin and the incidence of several chronic diseases, including cardiovascular diseases and cancer.

Methods

In June 1981 a detailed health questionnaire was sent to all residents of Leisure World, Laguna Hills, a retirement community near Los Angeles, California. New residents who moved into the community after this date were sent the questionnaire in June 1982, June 1983, and October 1985. Residents of this community are almost always white, moderately affluent, and well educated, and about two thirds are women.

The health questionnaire requested information on previous medical diagnoses, including angina, acute myocardial infarction, hypertension, rheumatoid arthritis, and diabetes; height and weight; the use of drugs, including analgesics, laxatives, and vitamin supplements; the use of cigarettes and alcohol; exercise habits; dietary intake of certain foods; and for women menstrual and reproductive events, including use of oestrogen replacement treatment. The use of analgesics was ascertained from the questions: "Which of the following best describes your use of non-prescription pain medication? Several times a day, daily, weekly, monthly, less often or never" and "When you take non-prescription pain medication, what is the brand (for example, Empirin, Anacin, Bayer Aspirin) you usually take?"

The cohort was followed up for all hospital admissions to three hospitals serving the area (85% of these occur at one hospital immediately adjacent to the community) and for deaths with the death certificate records of the local health department. Death certificates were also obtained for those who had died and been identified by the community business office, from the obituary column of the local newspaper, and from information provided by relatives and friends. In addition, we conducted two postings of follow up questionnaires to the cohort in 1983 and 1985. To date, only 13 members of the cohort have been lost to follow up; search of the National Death Index (a computerised register of death record information maintained by the National Center for Health Statistics) did not show that these people had died.

The members of the cohort were followed up to the time of the event of interest (admission to hospital) or death or to 1 January 1988, whichever came first. Age

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Br Med J 1988;297:1147-50

adjusted relative risks using five age groups (<75, 75-79, 80-84, 85-89, ≥90) and p values were obtained with a regression method that assumed that the development of cancer or chronic disease could be regarded as a Poisson process with a constant hazard rate for a given person.[11] The generalised linear interactive modelling (GLIM) software package program was used to make these calculations.[4] All reported p values are two sided.

Results

The residents' median age was 73. After three postings 13 987 residents (61% of the 22 781 residents) returned questionnaires; 8881 of the respondents were women. The distribution of sex was comparable in respondents and non-respondents, but the non-respondents were slightly older; overall mortality was greater in non-respondents in the first few years of follow up and then became identical with that in the respondents.

By 1 January 1988 the 8881 women and 5106 men in the cohort had contributed over 42 000 and 25 000 person years of follow up, respectively. The incidence of kidney cancer was increased among daily users of aspirin in both men and women, although the result was significant only in the men (table I). This increase was more evident for renal cell carcinomas (sex and age adjusted relative risk = 6·8, 95% confidence interval 2·0 to 20, $p<0·01$, for daily aspirin users compared with non-users) than for transitional cell cancers (relative risk = 2·2, 0·65 to 7·6, $p=0·20$).

Colon cancer showed some increased risk with the use of aspirin in both sexes (table I). The result was significant in both sexes combined (sex and age adjusted relative risk = 1·5, 1·1 to 2·2, $p<0·05$ for daily aspirin users compared with non-users). The increased risk was not restricted to a particular segment of the colon (data not shown). No other strong or significant cancer associations were apparent between the regular use of aspirin and the development of any other cancer.

TABLE I—*Numbers of cases and age adjusted relative risks for incidence of cancer by sex and use of analgesics containing aspirin*

Aspirin use	Colon: No of cases	Colon: Relative risk	Lung: No of cases	Lung: Relative risk	Bladder: No of cases	Bladder: Relative risk	Kidney: No of cases	Kidney: Relative risk	Prostate or breast: No of cases	Prostate or breast: Relative risk
Men										
None (n=3490)	47	1·00	46	1·00	55	1·00	6	1·00	106	1·00
<Daily (n=685)	13	1·09	5	0·57	6	0·77			18	0·90
Daily (n=870)	19	1·67	15	1·35	15	1·12	9	6·28*	22	0·75
Women										
None (n=6021)	66	1·00	22	1·00	14	1·00	6	1·00*	148	1·00
<Daily (n=1417)	15	0·95	6	1·00	3	1·56	1	0·72	23	0·95
Daily (n=1393)	21	1·41	2	0·29	3	0·89	3	2·11	23	0·94

*p<0·05.

TABLE II—*Numbers of cases and age adjusted relative risks for incidence of cardiovascular disease (excluding people with reported history of angina, acute myocardial infarction, and stroke) by sex and use of analgesics containing aspirin*

Aspirin use	Total (ICD 390-459): No of cases	Total (ICD 390-459): Relative risk	Stroke (ICD 430, 431, 434, 436): No of cases	Stroke (ICD 430, 431, 434, 436): Relative risk	Acute myocardial infarction (ICD 410): No of cases	Acute myocardial infarction (ICD 410): Relative risk	Ischaemic heart disease (ICD 411-414): No of cases	Ischaemic heart disease (ICD 411-414): Relative risk	Other heart disease (ICD 402, 424, 428, 429, 440, 441): No of cases	Other heart disease (ICD 402, 424, 428, 429, 440, 441): Relative risk
Men										
None (n=3367)	466	1·00	101	1·00	98	1·00	54	1·00	87	1·00
<Daily (n=737)	88	1·02	16	0·87	13	0·84	17	1·51	21	1·23
Daily (n=816)	113	1·44*	28	1·33	14	0·78	19	1·68*	29	1·66
Women										
None (n=5169)	368	1·00	135	1·00	56	1·00	77	1·00	124	1·00
<Daily (n=1267)	122	0·97	29	0·88	22	1·16	25	1·45	33	0·85
Daily (n=1062)	146	1·39*	37	1·17	20	1·06	28	1·72*	33	1·31

*p<0·05.
ICD = International Classification of Diseases (ninth revision).

For analysis of the relation between cardiovascular disease and use of aspirin we excluded 1432 men and 1894 women who had reported a history of angina, myocardial infarction, or stroke in the initial survey (table II). The incidence of ischaemic heart disease was raised among daily users of aspirin compared with non-users among both men and women (relative risk = 1·9, 1·1 to 3·1, $p<0·05$ and 1·7, 1·1 to 2·7, $p<0·05$, respectively). Except for "other heart disease" (including various diseases, the numerically most important of which was heart failure) in men, the use of aspirin was not significantly related to any other vascular disease examined. A 20-30% increased risk of stroke was, however, seen in men and women who took aspirin daily, but neither of these results was significant (sex and age adjusted relative risk = 1·3, 0·96 to 1·7, $p=0·10$ for daily aspirin users compared with non-users). For all cardiovascular disease combined the risk was raised among daily users of aspirin compared with non-users among both men and women (relative risks = 1·5, 1·2 to 1·8, $p<0·05$ and 1·4, 1·2 to 1·7, $p<0·05$, respectively).

Other chronic diseases examined because of previous evidence of a possible association with regular use of aspirin included gastrointestinal bleeding, gastric ulcers, and cataracts (table III). Among women, those who used aspirin less than daily showed a significant reduction in risk for ulcers (relative risk = 0·5, 0·14 to 0·74, $p<0·05$), but there was no evidence of a dose-response effect. No other result was significant or substantially different from unity.

TABLE III—*Numbers of cases and age adjusted relative risks for incidence of other diseases by sex and use of analgesics containing aspirin*

Aspirin use	Gastrointestinal bleeding: No of cases	Gastrointestinal bleeding: Relative risk	Ulcers: No of cases	Ulcers: Relative risk	Cataracts: No of cases	Cataracts: Relative risk
Men						
None (n=3499)	37	1·00	33	1·00	120	1·00
<Daily (n=685)	5	0·73	13	1·56	21	0·83
Daily (n=870)	13	1·88	13	1·00	39	1·21
Women						
None (n=6021)	57	1·00	81	1·00	848	1·00
<Daily (n=1417)	8	0·95	6	0·52*	63	0·89
Daily (n=1393)	12	1·29	5	0·76	99	1·22

*p<0·05.

Discussion

Five case-control studies of cancer of the renal pelvis[5,6] and two of renal cell carcinoma[7,8] have evaluated the association with use of analgesics, and we have reviewed the subject.[9] Two studies conducted by McCredie *et al* in New South Wales suggested a strong association between "regular" use of analgesics and cancer of the renal pelvis in both sexes.[5,7] The second study suggested that in women this strong association was limited mainly to compounds containing phenacetin.[7] In a study in Minnesota McLaughlin *et al* found 3·9-fold and 3·7-fold excess risks of cancer of the renal pelvis in men and women, respectively, who were regular long term (>36 months) users of drugs containing phenacetin or paracetamol compared with non-users.[6] Neither result, however, was significant. Our case-control study of cancer of the renal pelvis and ureter suggested that heavy use of analgesics obtained over the counter without a prescription is associated with an increased risk and that the increased risk extends across formulations containing all the main active ingredients of preparations currently used in the United States, including aspirin.[9] The data on renal cell carcinoma are scanty and inconclusive.[8,10] Our current results are compatible with these published findings for cancer of the renal pelvis and suggest that